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(54) **COMPOSITIONS COMPRISING HMW-MAA AND FRAGMENTS THEREOF, AND METHODS OF USE THEREOF**

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(57) **ABSTRACT**

This invention provides recombinant polypeptides comprising a fragment of a High Molecular Weight Melanoma-Associated Antigen (HMW-MAA), recombinant *Listeria* strains comprising same, and methods of inducing an immune response and treating and impeding the growth of tumors, comprising administering same.

9 Claims, 16 Drawing Sheets

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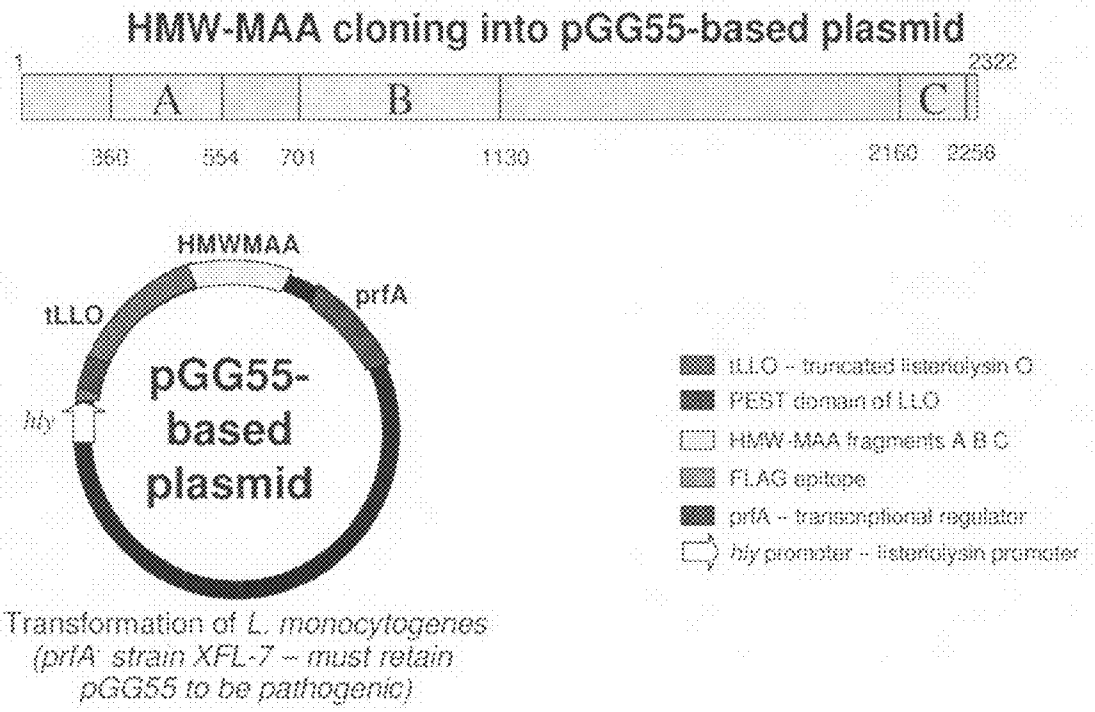

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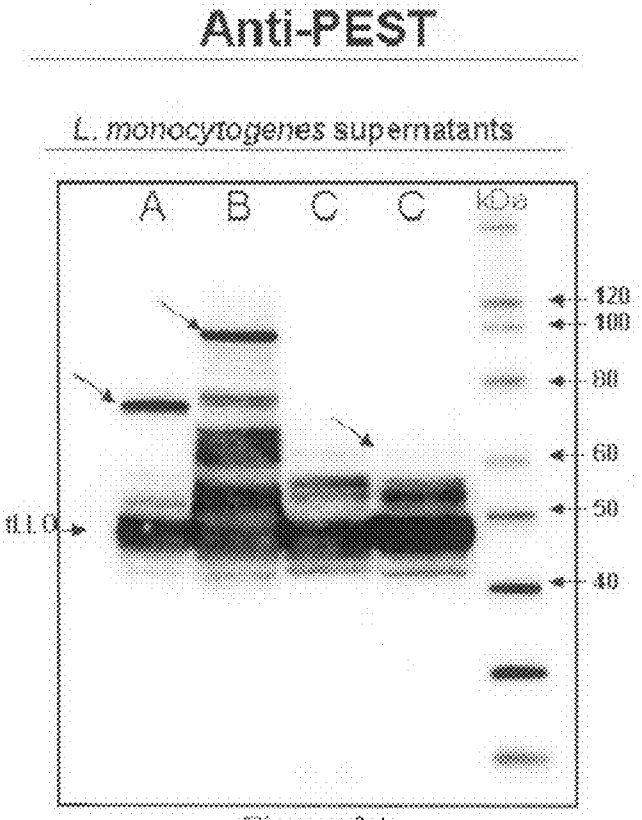

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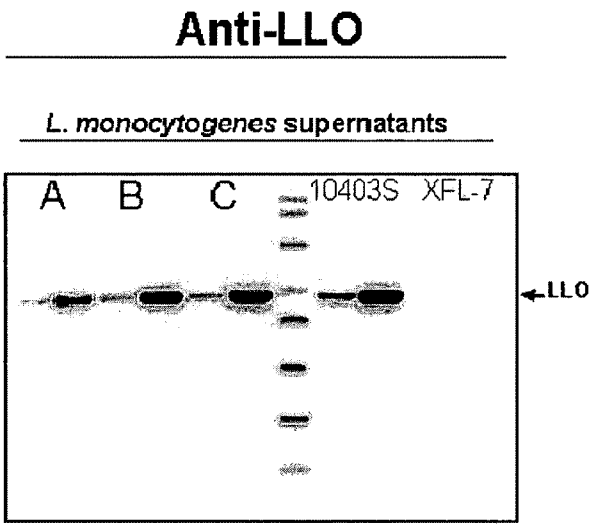

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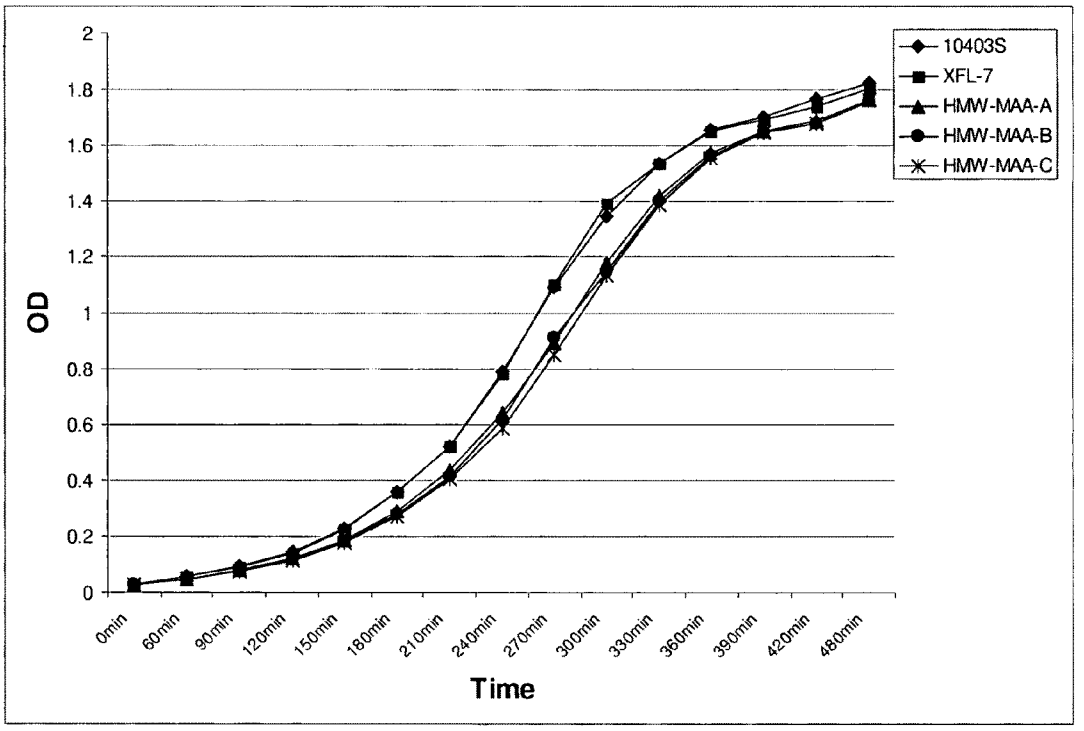

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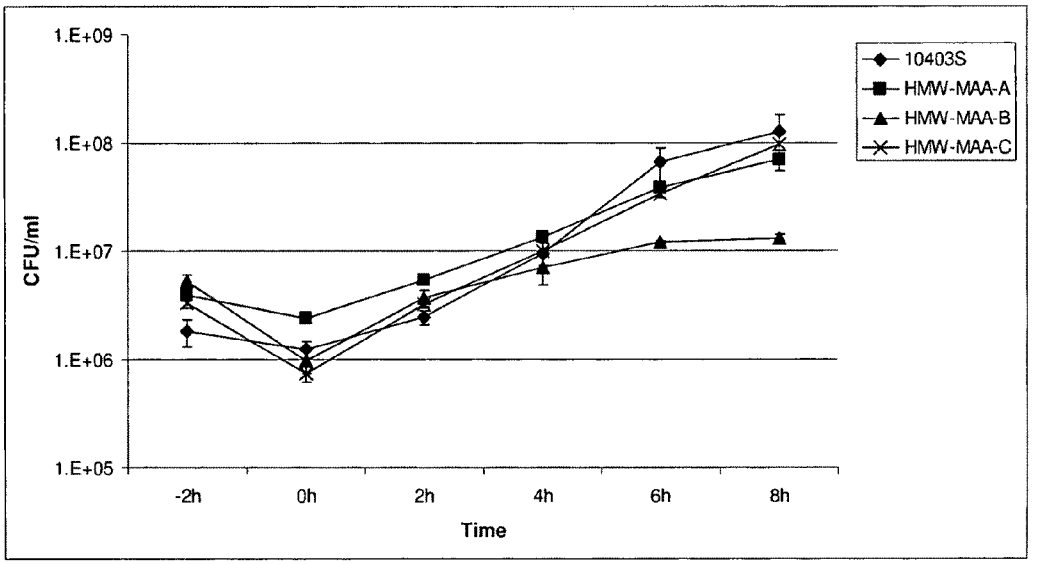
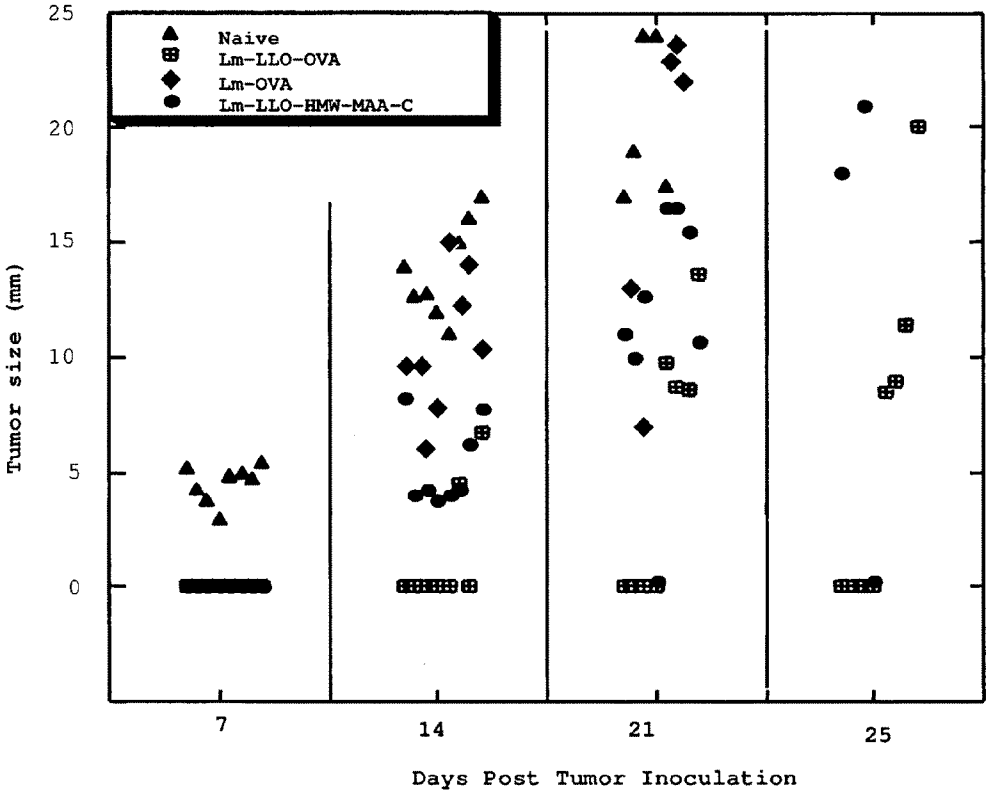


Figure 3B



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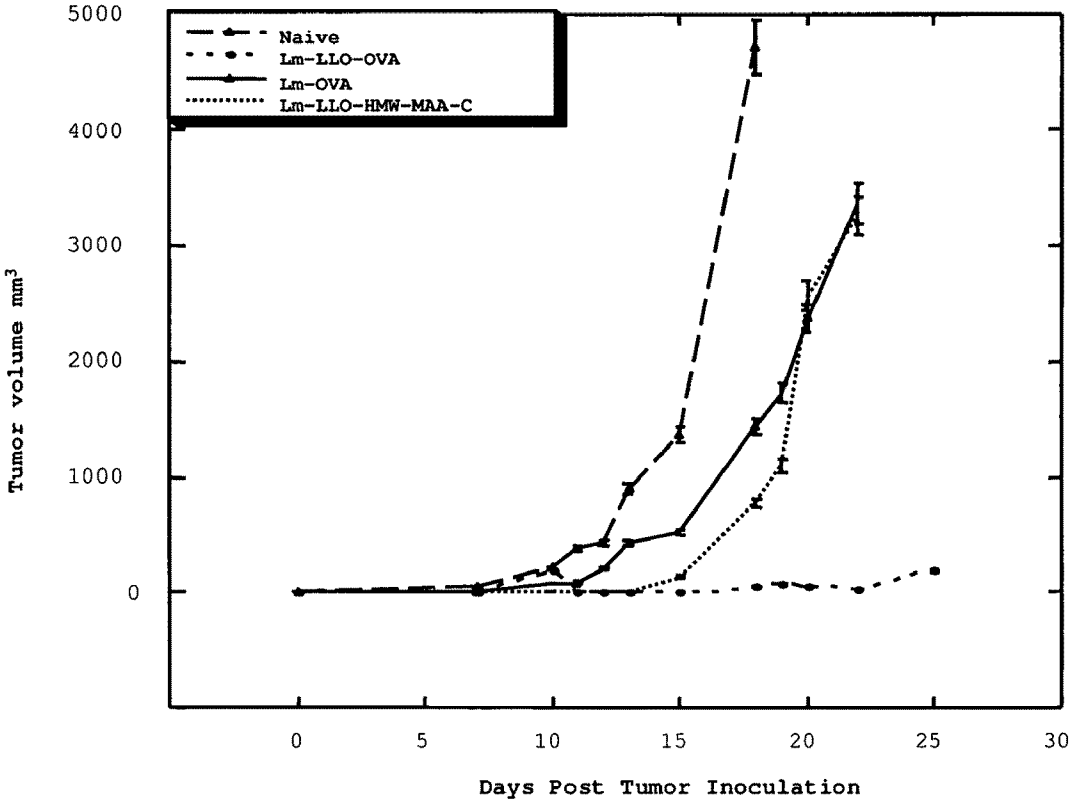

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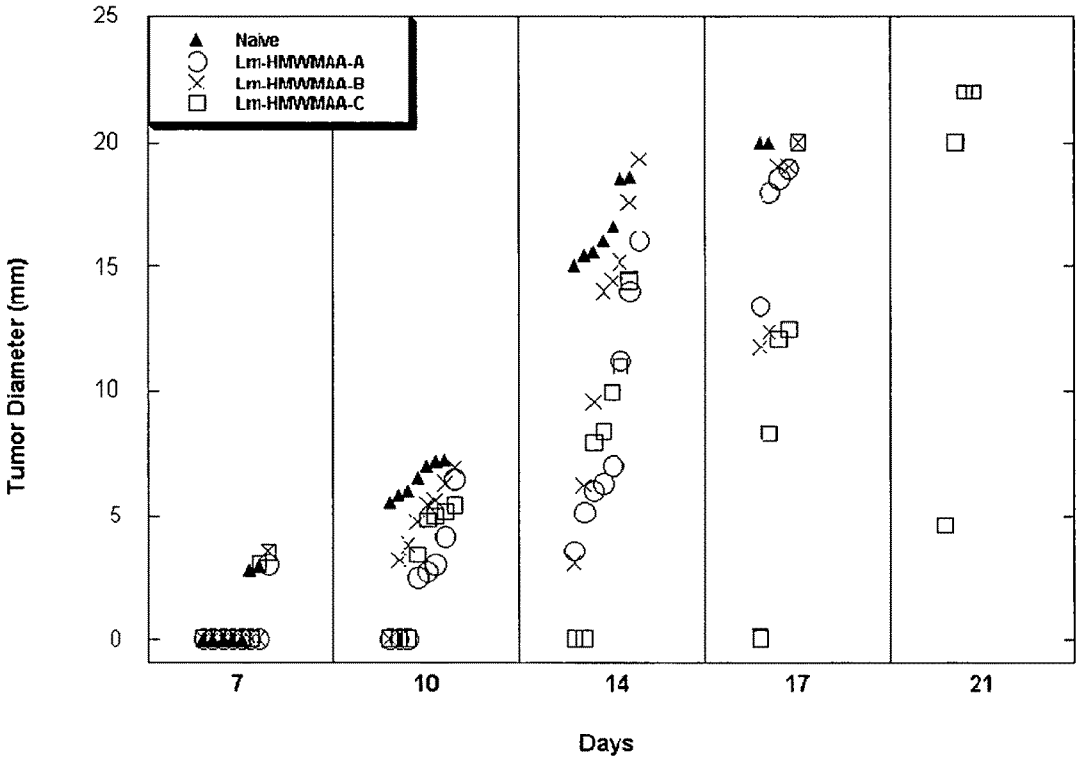

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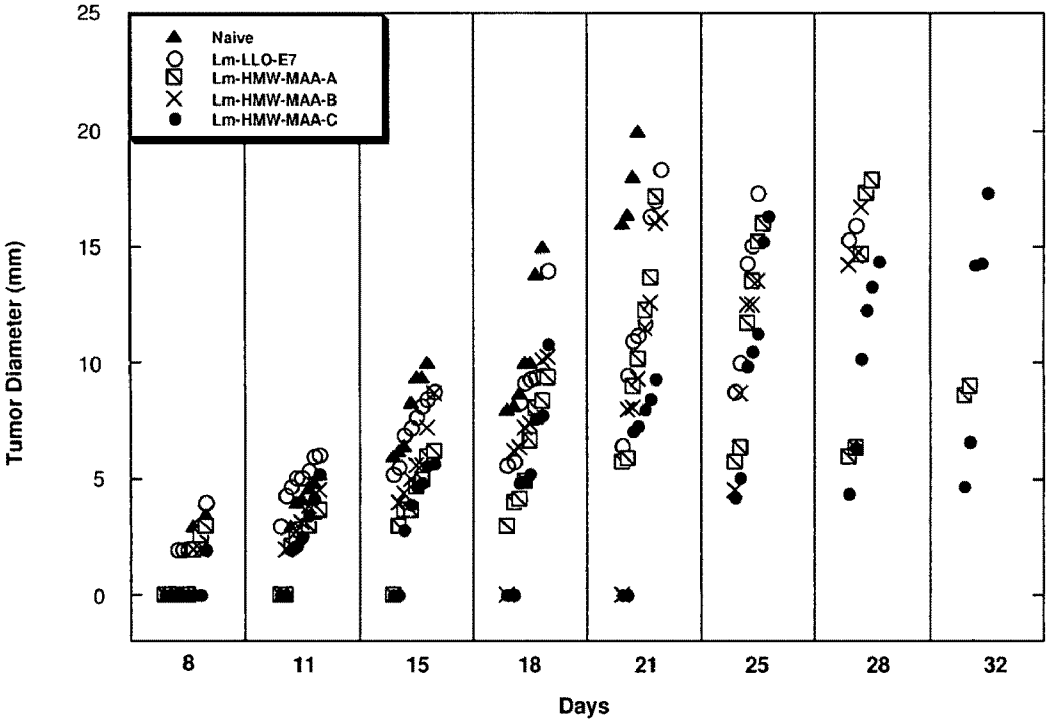
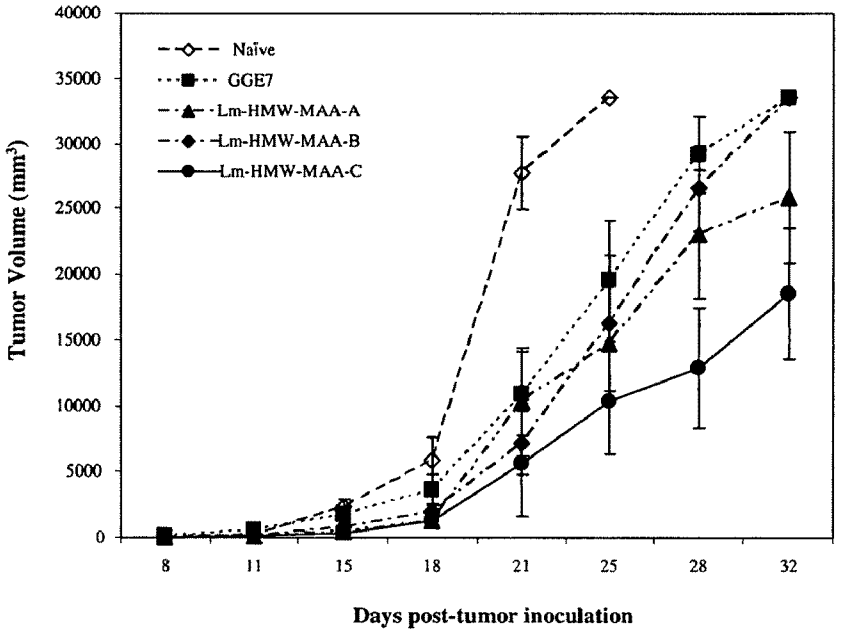


Figure 4D



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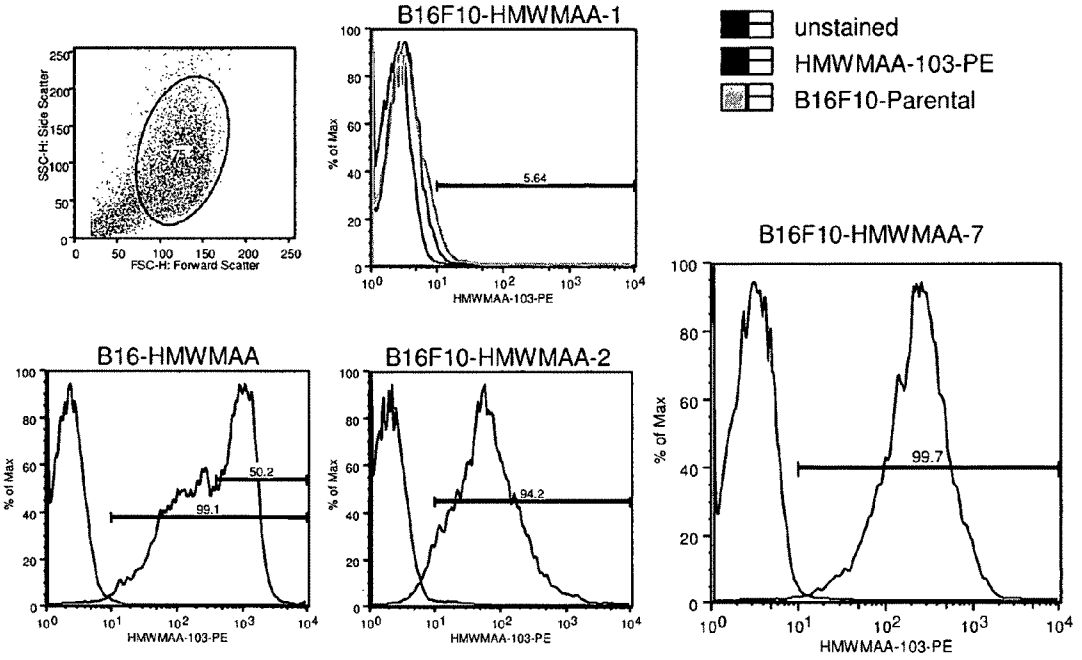

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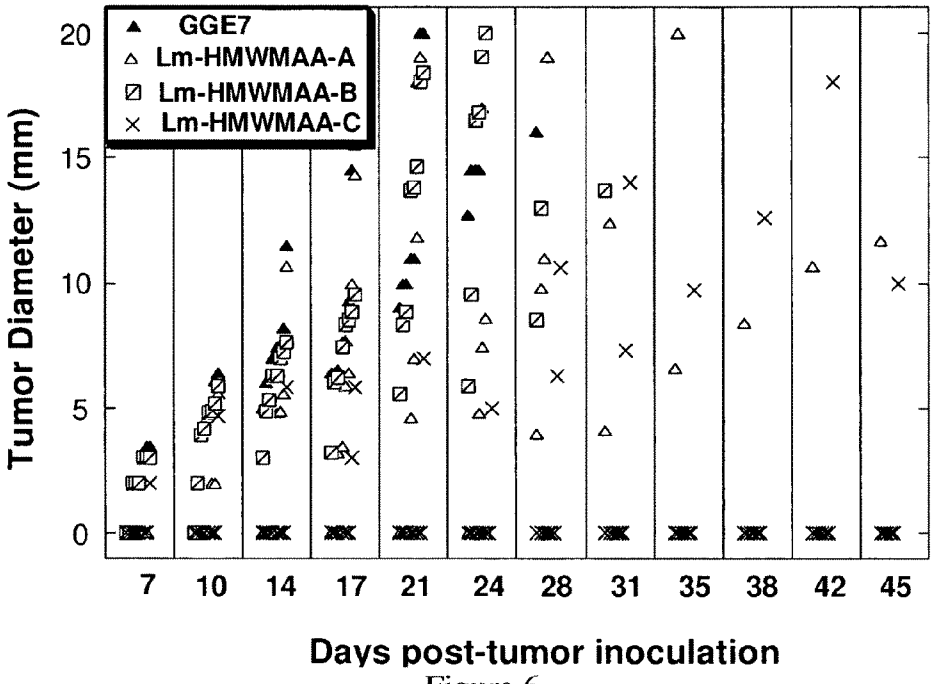

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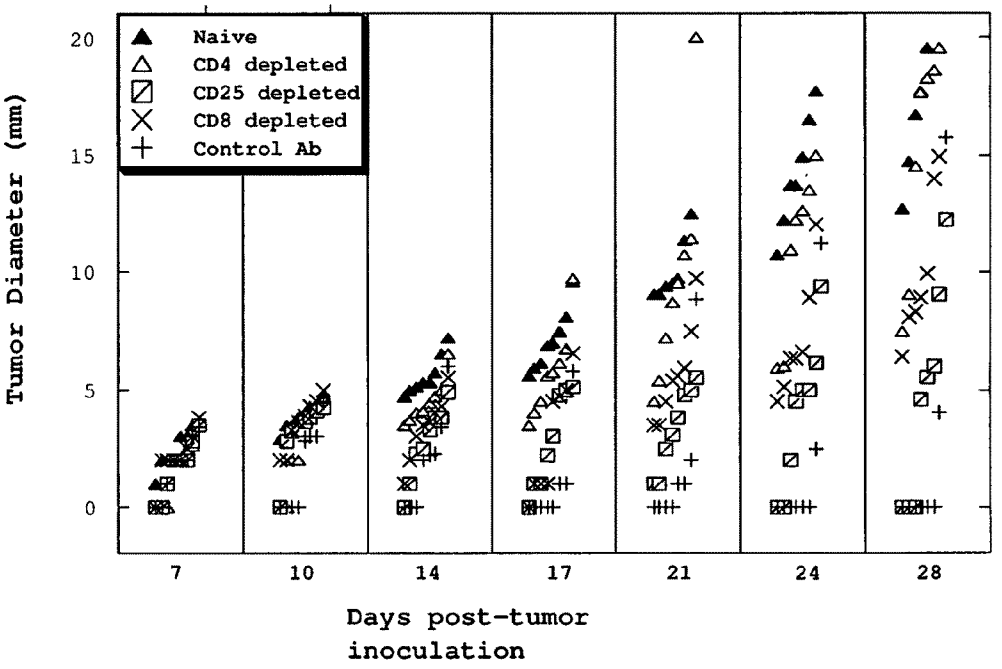

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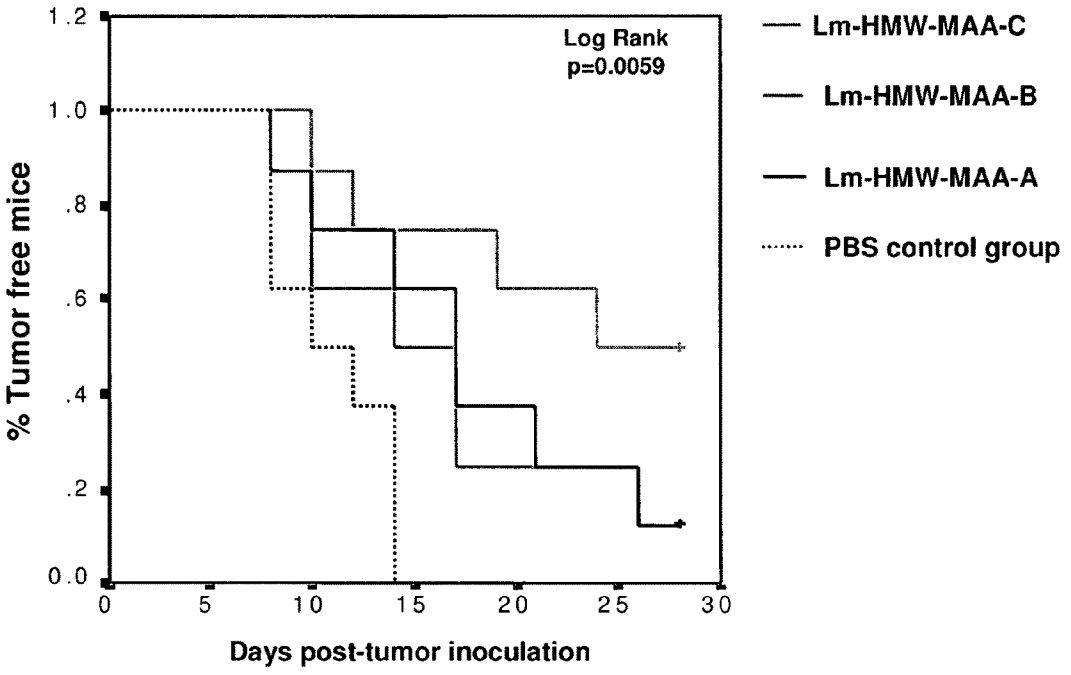

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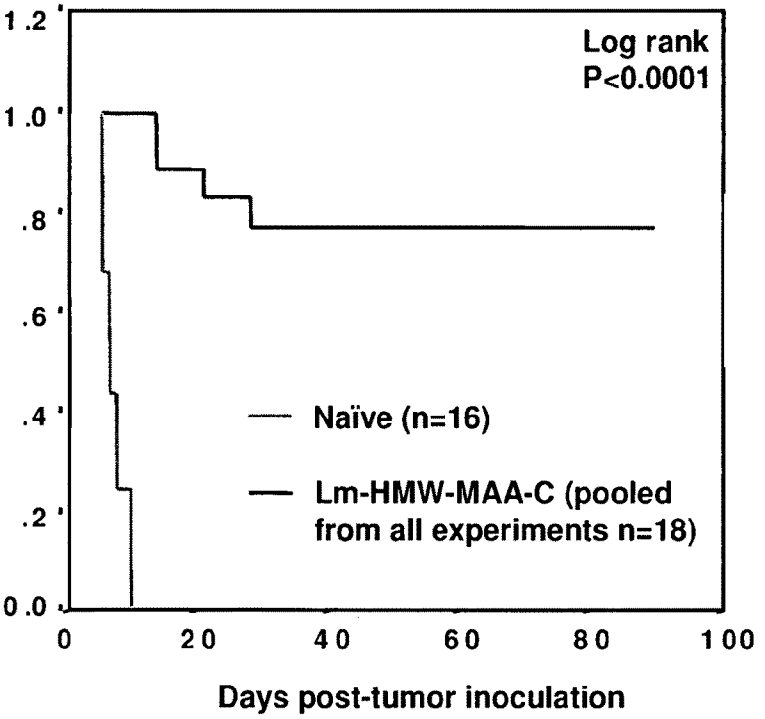

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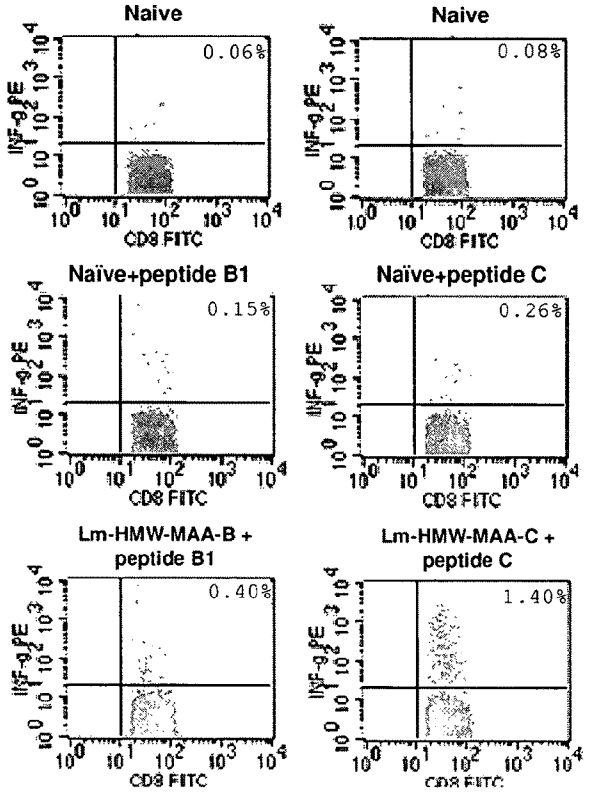


Figure 10A

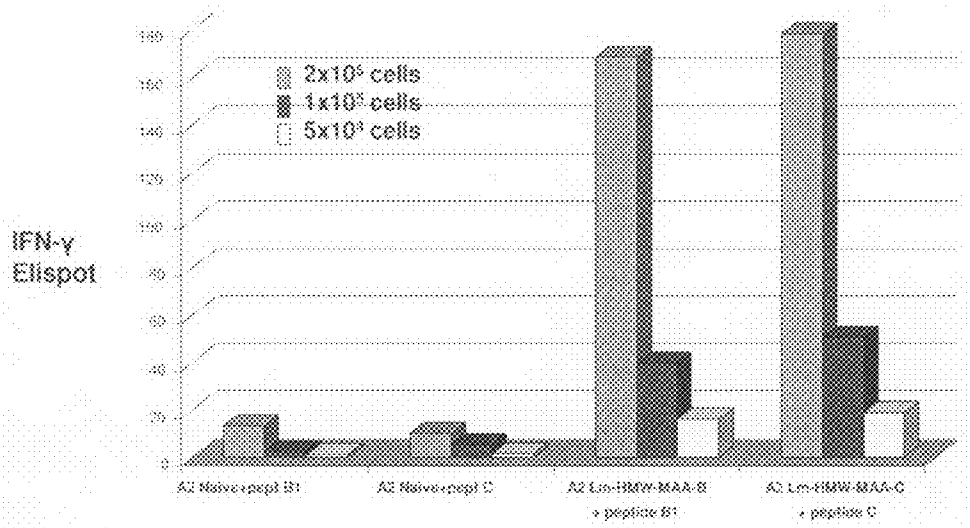


Figure 10B

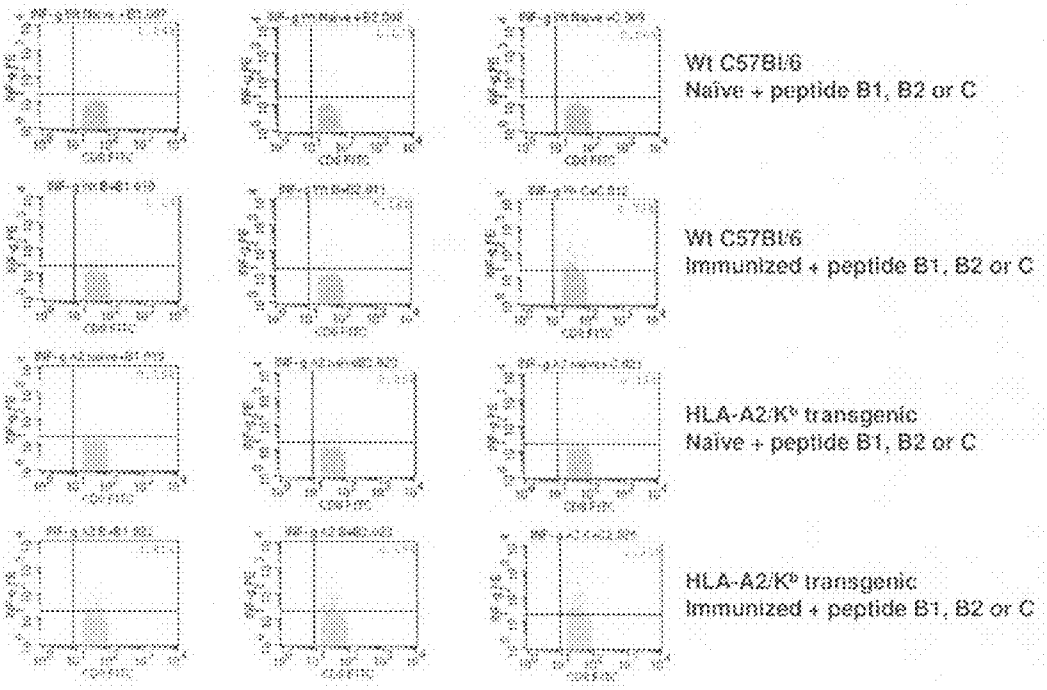


Figure 11A

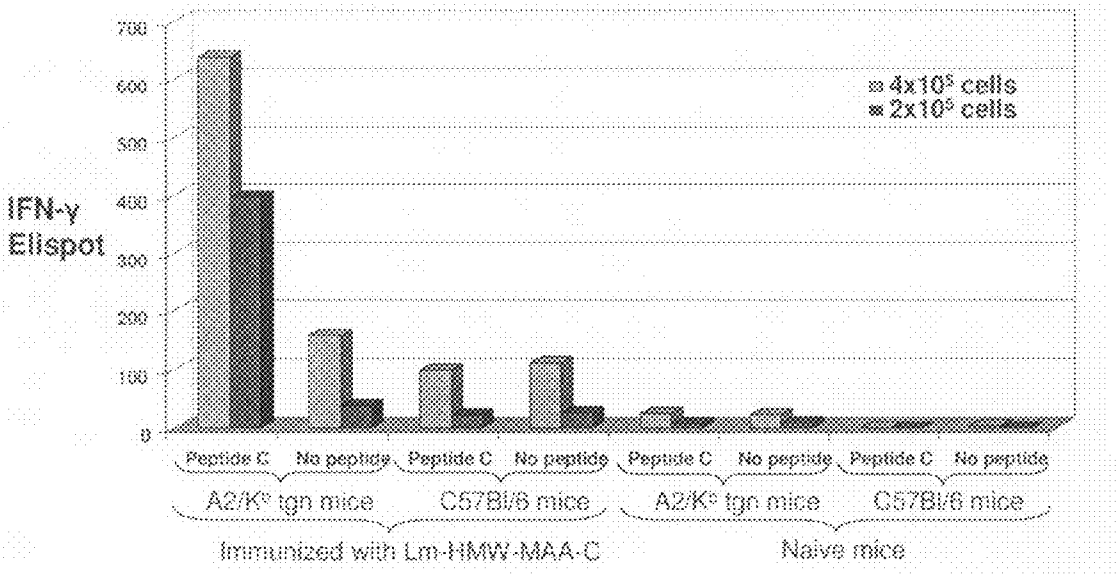
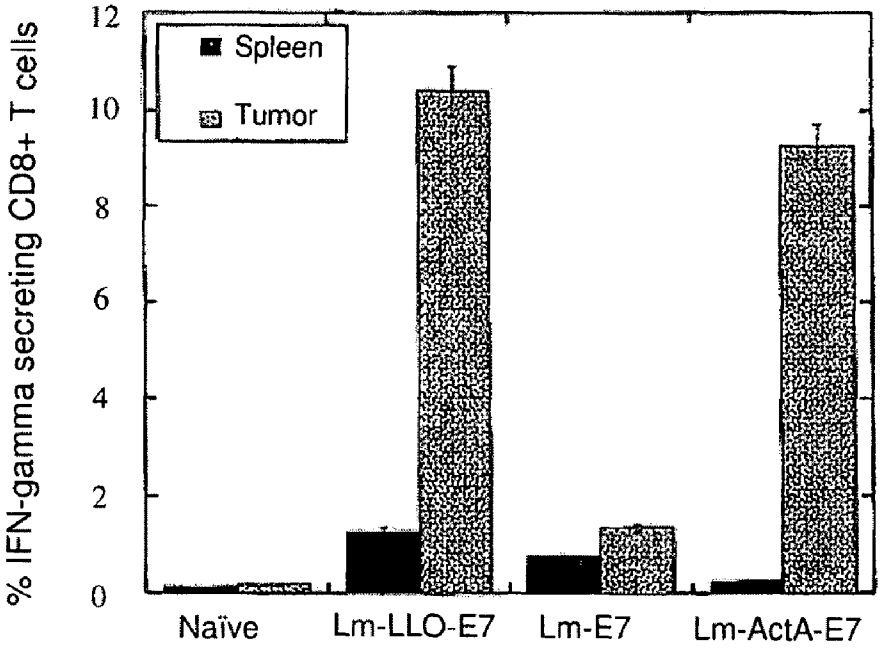

IFN-γ Elispot
700
600
500
400
300
200
100
0
4x10^5 cells
2x10^5 cells
Peptide C
No peptide
Peptide C
No peptide
Peptide C
No peptide
Peptide C
No peptide
A2/K^b tgn mice
C57Bl/6 mice
A2/K^b tgn mice
C57Bl/6 mice
Immunized with Lm-HMW-MAA-C
Naive mice

Figure 11B



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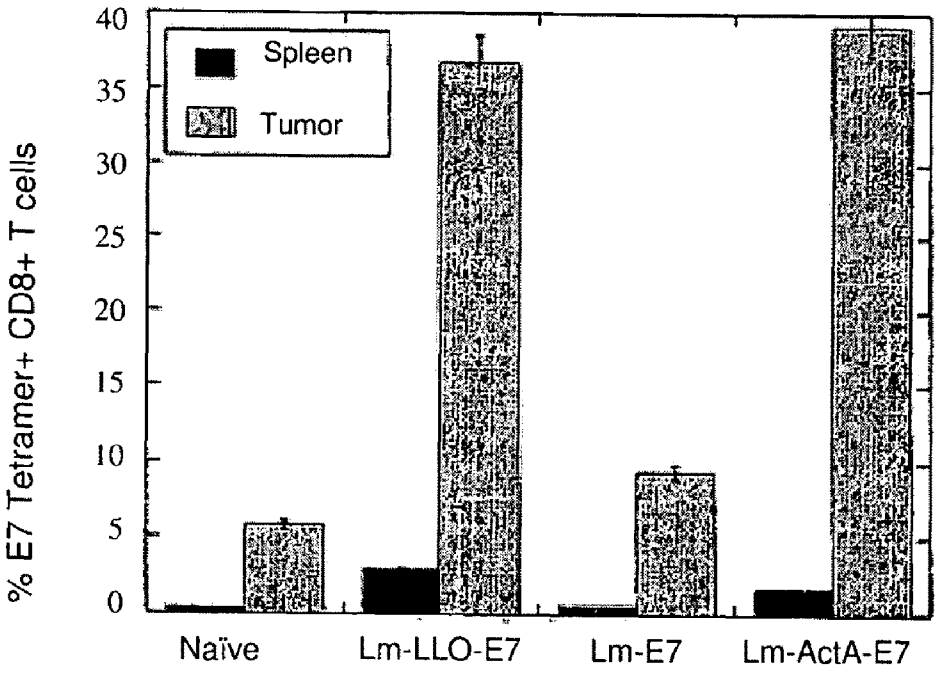

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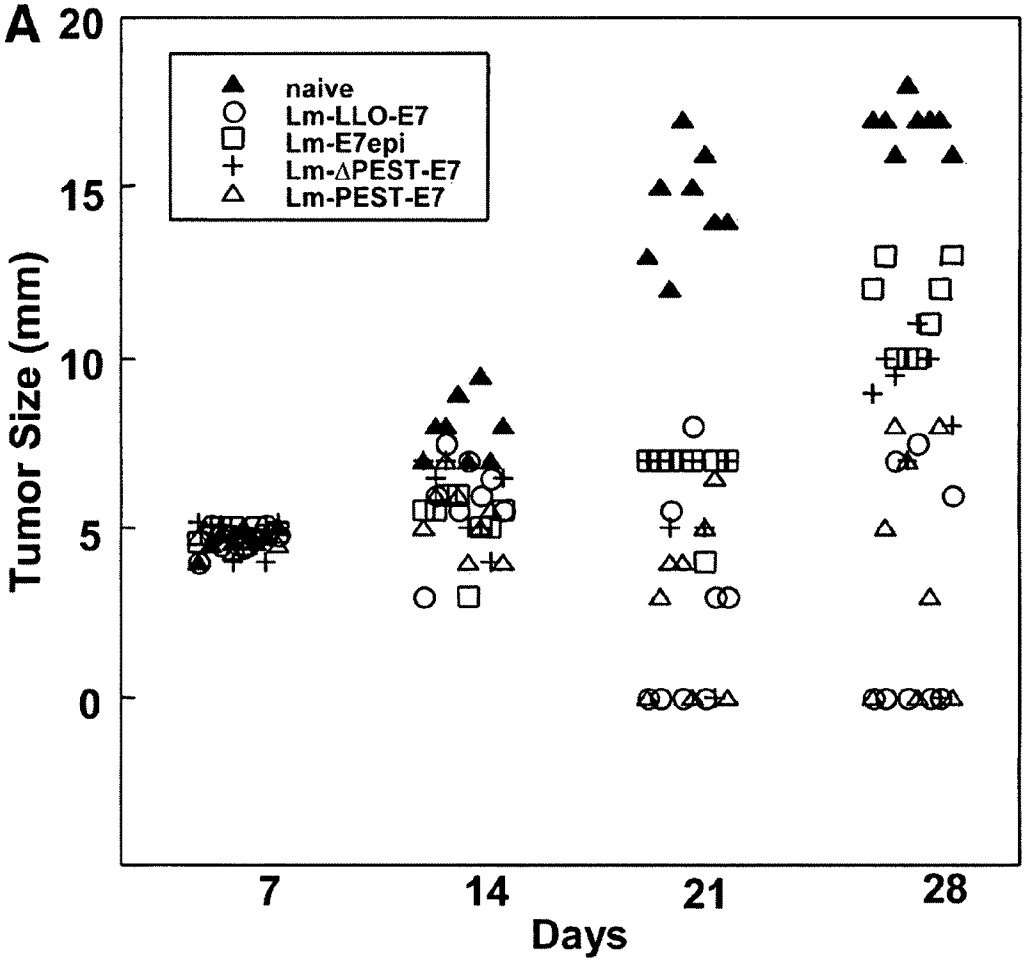

A
naive
Lm-LLO-E7
Lm-E7epi
Lm-ΔPEST-E7
Lm-PEST-E7
Tumor Size (mm)
0
5
10
15
20
7
14
21
28
Days

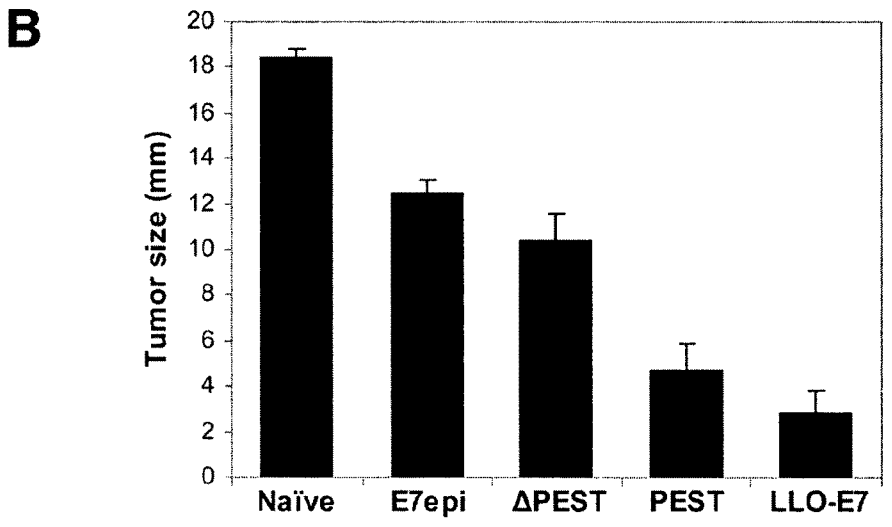
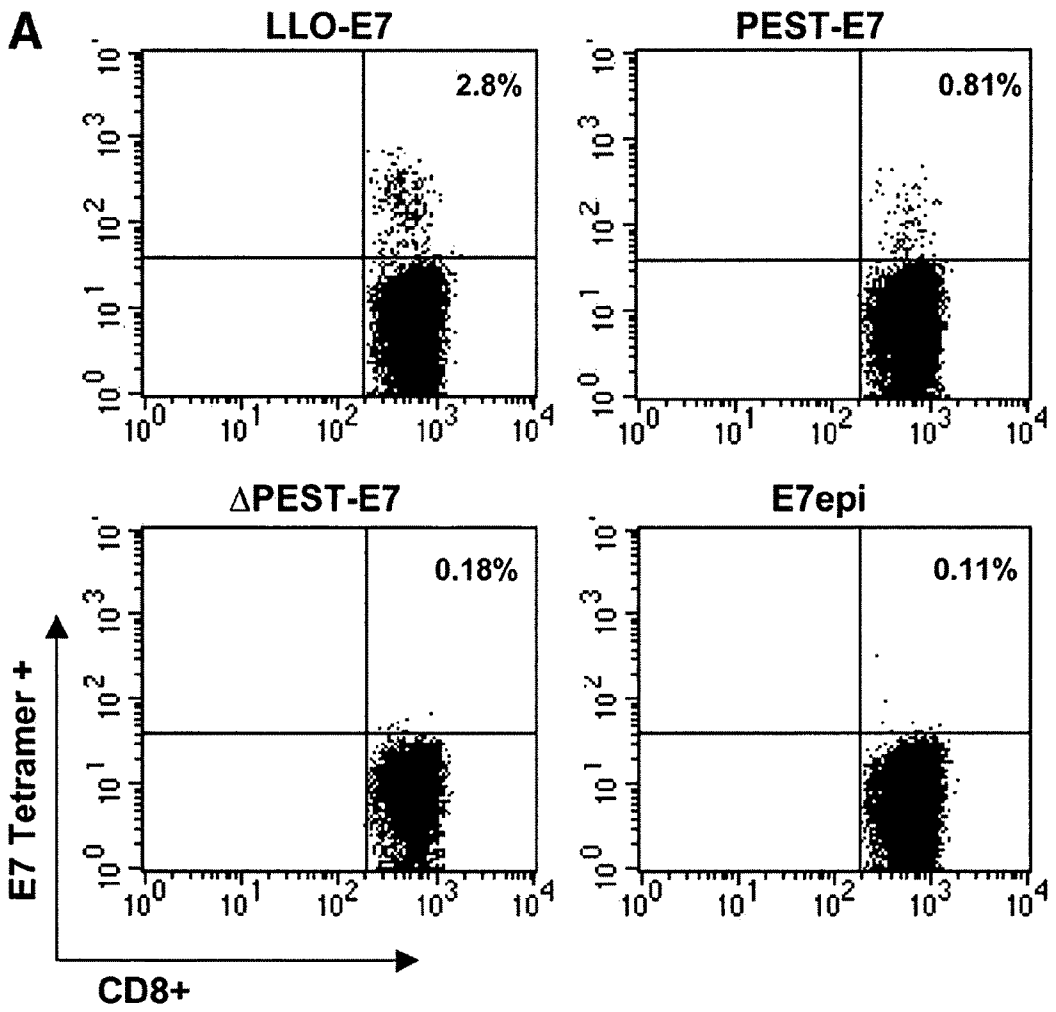

B
Tumor size (mm)
0
2
4
6
8
10
12
14
16
18
20
Naïve
E7epi
ΔPEST
PEST
LLO-E7

Figure 14


A
LLO-E7
2.8%
PEST-E7
0.81%
ΔPEST-E7
0.18%
E7epi
0.11%
E7 Tetramer +
CD8+

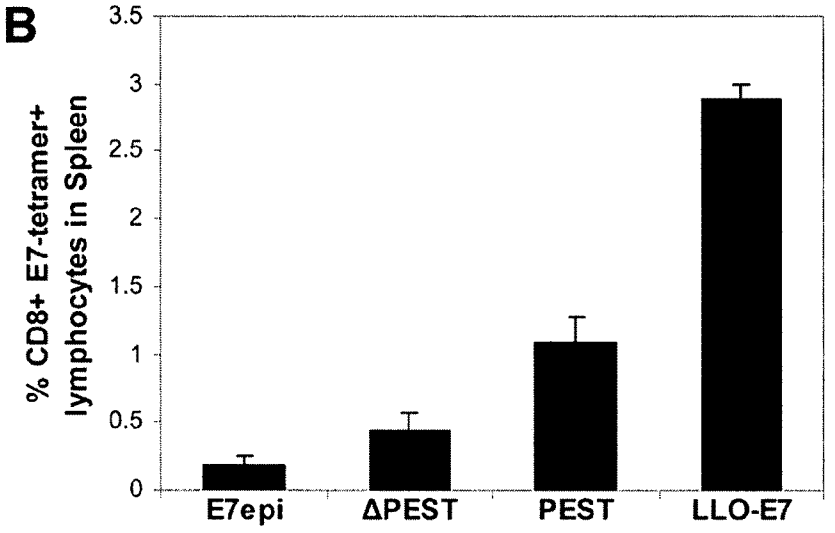

B
% CD8+ E7-tetramer+ lymphocytes in Spleen
3.5
3
2.5
2
1.5
1
0.5
0
E7epi
ΔPEST
PEST
LLO-E7

Figure 15

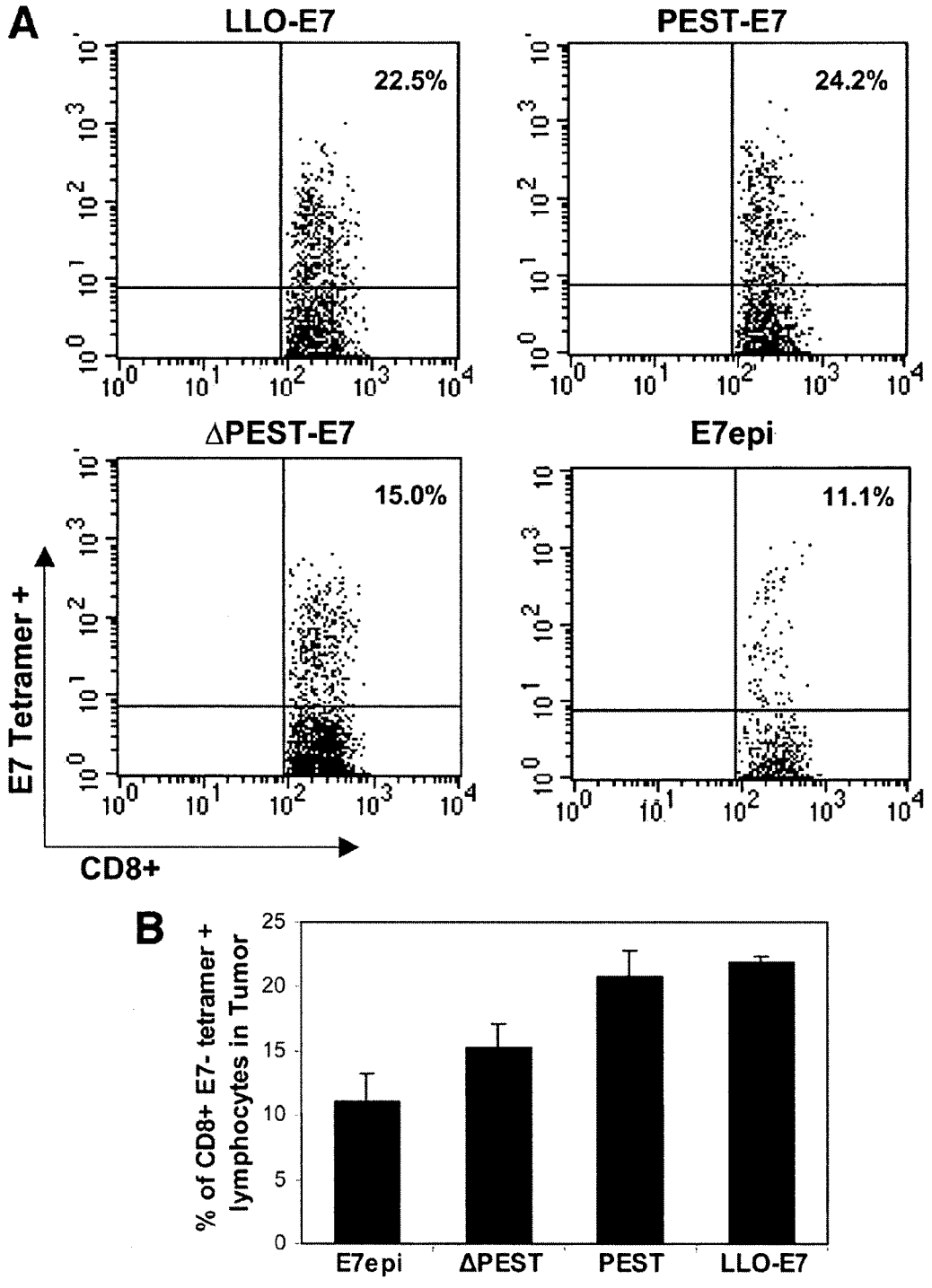

A
LLO-E7
22.5%
PEST-E7
24.2%
ΔPEST-E7
15.0%
E7epi
11.1%
E7 Tetramer +
CD8+
B
% of CD8+ E7- tetramer + lymphocytes in Tumor
25
20
15
10
5
0
E7epi
ΔPEST
PEST
LLO-E7

Figure 16

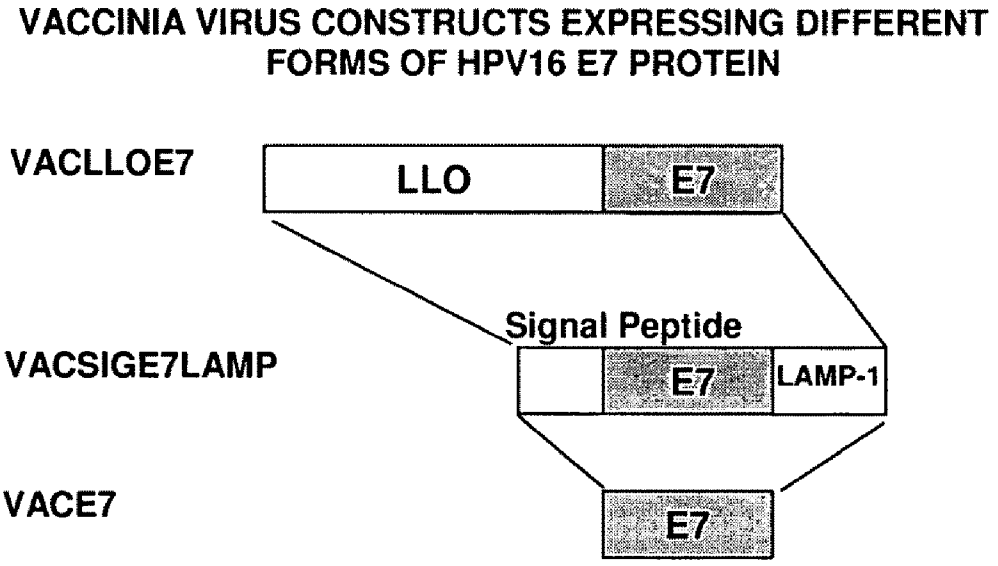

VACCINIA VIRUS CONSTRUCTS EXPRESSING DIFFERENT FORMS OF HPV16 E7 PROTEIN
VACLLOE7
LLO
E7
Signal Peptide
VACSIGE7LAMP
E7
LAMP-1
VACE7
E7

Figure 17

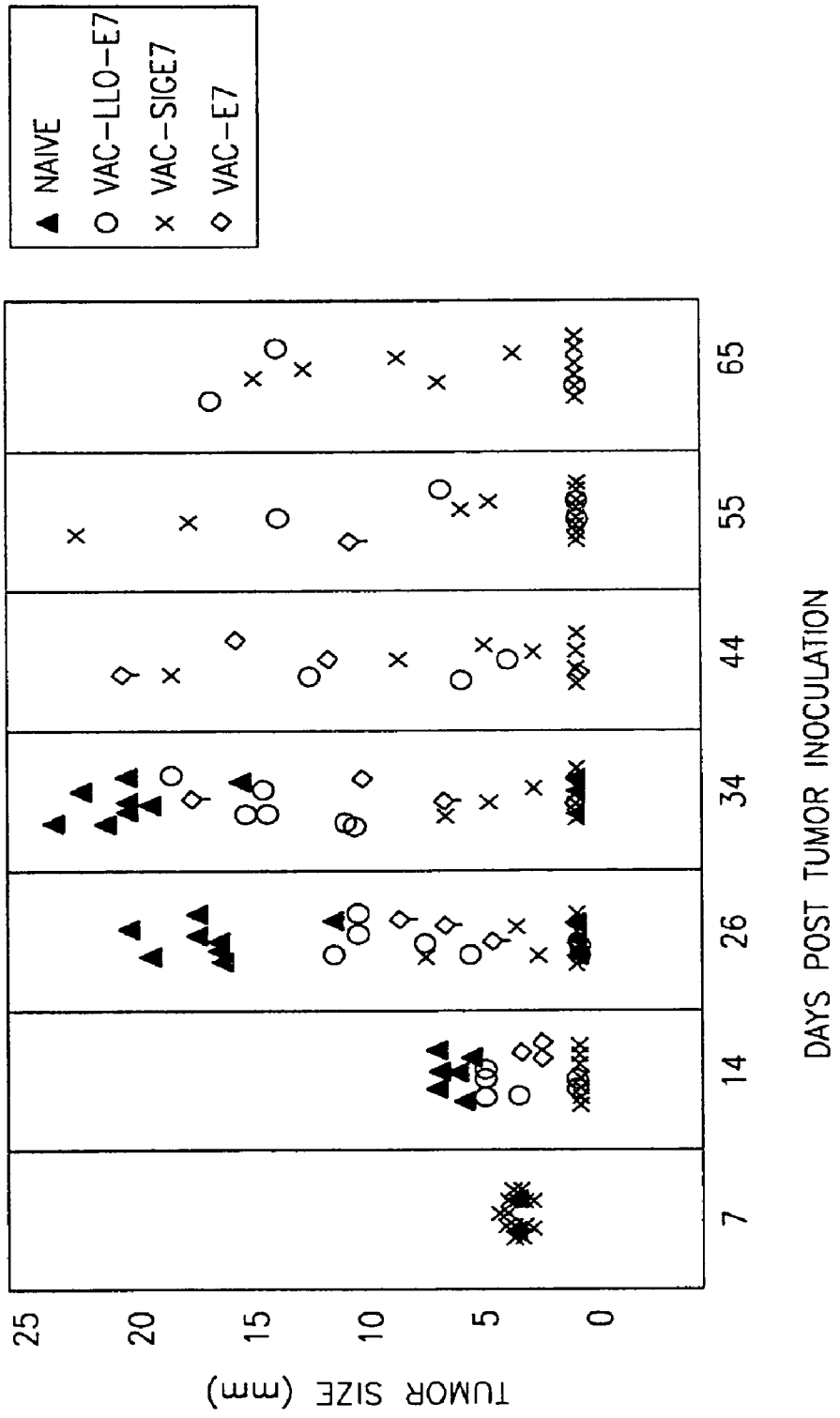

NAIVE
VAC-LLO-E7
VAC-SIGE7
VAC-E7
TUMOR SIZE (mm)
0
5
10
15
20
25
7
14
26
34
44
55
65
DAYS POST TUMOR INOCULATION

Figure 18

COMPOSITIONS COMPRISING HMW-MAA AND FRAGMENTS THEREOF, AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from U.S. Provisional Application Ser. No. 60/837,608 filed Aug. 15, 2006, which is incorporated in its entirety herein by reference.

FIELD OF INVENTION

This invention provides recombinant polypeptides comprising a fragment of a High Molecular Weight Melanoma-Associated Antigen (HMW-MAA), recombinant *Listeria* strains comprising same, and methods of inducing an immune response and treating and impeding the growth of tumors, comprising administering same.

BACKGROUND OF THE INVENTION

HMW-MAA, also known as the melanoma chondroitin sulfate proteoglycan (MCSP), is a transmembrane protein of 2322 residues. HMW-MAA is expressed on over 90% of surgically removed benign nevi and melanoma lesions, and is also expressed in basal cell carcinoma, tumors of neural crest origin (e.g. astrocytomas, gliomas, neuroblastomas and sarcomas), childhood leukemias, and lobular breast carcinoma lesions.

Treatments and cures for many tumors e.g. HMW-MAA-expressing tumors, as well as methods for prevention especially in high risk populations, are urgently needed in the art.

SUMMARY OF THE INVENTION

This invention provides in some embodiments, recombinant polypeptides comprising a fragment of a High Molecular Weight-Melanoma Associated Antigen (HMW-MAA), recombinant *Listeria* strains comprising same, and methods of inducing an immune response and treating and impeding the growth of tumors, comprising administering same.

In one embodiment, the present invention provides a recombinant *Listeria* strain comprising a recombinant polypeptide, the recombinant polypeptide comprising a fragment of a HMW-MAA protein ("HMW-MAA fragment").

In another embodiment, the present invention provides a recombinant polypeptide comprising a fragment of a HMW-MAA protein operatively linked to a non-HMW-MAA oligopeptide selected from a listeriolysin (LLO) oligopeptide, an ActA oligopeptide, or a PEST-like oligopeptide or homologues thereof.

In another embodiment, the present invention provides a recombinant polypeptide comprising a fragment of a HMW-MAA protein, wherein the fragment consists of about amino acids (AA) 360-554 of the HMW-MAA protein from which the fragment is derived. In another embodiment, the fragment consists of about AA 701-1130. In another embodiment, the fragment consists of about AA 2160-2258. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a vaccine comprising a recombinant *Listeria* strain of the present invention and an adjuvant.

In another embodiment, the present invention provides an immunogenic composition comprising a recombinant *Listeria* strain of the present invention.

In another embodiment, the present invention provides a vaccine comprising a recombinant polypeptide of the present invention.

In another embodiment, the present invention provides an immunogenic composition comprising a recombinant polypeptide of the present invention.

In another embodiment, the present invention provides a recombinant vaccine vector encoding a recombinant polypeptide of the present invention.

In another embodiment, the present invention provides a recombinant *Listeria* strain comprising a recombinant polypeptide of the present invention.

In another embodiment, the present invention provides a method of inducing an anti-HMW-MAA immune response in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby inducing an anti-HMW-MAA immune response in a subject.

In another embodiment, the present invention provides a method of delaying progression of a solid tumor in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby delaying progression of a solid tumor in a subject. In another embodiment, the subject mounts an immune response against a pericyte of the solid tumor. In another embodiment, the pericyte is in a vasculature of the solid tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of impeding vascularization of a solid tumor in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby impeding vascularization of a solid tumor in a subject. In another embodiment, the subject mounts an immune response against a pericyte of the solid tumor. In another embodiment, the pericyte is in a vasculature of the solid tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of delaying progression of a HMW-MAA-expressing tumor in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby delaying progression of a HMW-MAA-expressing tumor in a subject. In another embodiment, the subject mounts an immune response against the HMW-MAA-expressing tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of treating a HMW-MAA-expressing tumor in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby treating a HMW-MAA-expressing tumor in a subject. In another embodiment, the subject mounts an immune response against the HMW-MAA-expressing tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of inducing an anti-HMW-MAA immune response in a subject, comprising administering to the subject a composition comprising a recombinant polypeptide of the present invention, thereby inducing an anti-HMW-MAA immune response in a subject.

In another embodiment, the present invention provides a method of delaying progression of a solid tumor in a subject, comprising administering to the subject a composition comprising a recombinant polypeptide of the present invention, thereby delaying progression of a solid tumor in a subject. In

another embodiment, the subject mounts an immune response against a pericyte of the solid tumor. In another embodiment, the pericyte is in a vasculature of the solid tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of impeding a vascularization of a solid tumor in a subject, comprising administering to the subject a composition comprising a recombinant polypeptide of the present invention, thereby impeding a vascularization of a solid tumor in a subject. In another embodiment, the subject mounts an immune response against a pericyte of the solid tumor. In another embodiment, the pericyte is in a vasculature of the solid tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of delaying progression of a HMW-MAA-expressing tumor in a subject, comprising administering to the subject a composition comprising a recombinant polypeptide of the present invention, thereby delaying progression of a HMW-MAA-expressing tumor in a subject. In another embodiment, the subject mounts an immune response against the HMW-MAA-expressing tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of treating a HMW-MAA-expressing tumor in a subject, comprising administering to the subject a composition comprising a recombinant polypeptide of the present invention, thereby treating a HMW-MAA-expressing tumor in a subject. In another embodiment, the subject mounts an immune response against the HMW-MAA-expressing tumor. Each possibility represents a separate embodiment of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1**: HMW-MAA cloning into pGG55-based plasmid. Lm-LLO-HMW-MAA was generated by transforming the prfA$^-$ strain XFL-7 with the plasmid pGG-55. pGG-55 has the hly promoter driving expression of a non-hemolytic fusion of LLO-E7 and the prfA gene to select for retention of the plasmid. XFL-7 must retain the plasmid in order to be viable.

FIG. **2**. LLO-HMW-MAA constructs are expressed. Supernatant was harvested from LM strains transformed with the LLO-HMW-MAA A, B and C plasmids. A. Anti-PEST probes revealed that all three strains produced fusion proteins of the expected sizes (48 Kda for LLO, 75 Kda for HMW-MAA-A, 98 Kda for HMW-MAA-B, and 62 Kda for HMW-MAA-C). B. Anti-LLO probes revealed LLO bands for HMW-MAA-A, HMW-MAA-B, HMW-MAA-C, and in 10403S controls.

FIG. **3**. *Listeria* strains expressing LLO-HMW-MAA constructs exhibit growth in media (A), virulence, and intracellular growth (B) similar to wild type Lm.

FIG. **4**. HMW-MAA-expressing Lm impedes the growth of tumors, even in tumor cells that do not express HMW-MAA. 10^8 cfu of Lm-HMW-MAA-C impedes B 16F0-Ova tumor growth as measured by tumor size (A) and volume (B) significantly compared to the naïve group. Similar effects on tumor diameter and volume were observed with all three Lm-LLO-HMW-MAA strains after inoculation of C57BL/6 mice with B16F0-Ova (C) and RENCA (D and E) tumor cells.

FIG. **5**. Selection of HMW-MAA-expressing B16F10 murine tumor cell clones.

FIG. **6**. Lm-HMW-MAA constructs induced antigen-specific immune responses that impede tumor growth.

FIG. **7**. In vivo depletion of either CD4+ or CD8+ cells abrogated the efficacy of Lm-HMW-MAA-C vaccine.

FIG. **8**. CD8+ T cells from mice vaccinated with Lm-HMW-MAA-C mice inhibited the growth of B16F10 HMW-MAA tumors in vivo.

FIG. **9**. Mice vaccinated with Lm-HMW-MAA-C that eliminated the B 16F10-HMW-MAA tumor were protected against a second challenge with the same tumor.

FIG. **10**. Immunization of HLA-A2/Kb transgenic mice with Lm-HMW-MAA-B and Lm-HMW-MAA-C induced detectable immune responses against two characterized HMW-MAA HLA-A2 epitopes in fragments B and C.

FIG. **11**. IFN-γ secretion by T cells stimulated with an HLA-A2 restricted peptide from fragment C of HMW-MAA after one immunization with Lm-HMW-MAA-C in HLA-A2/Kb and wild-type C57B1/6 mice.

FIG. **12**. Depiction of vaccinia virus constructs expressing different forms of HPV16E7 protein.

FIG. **13**. Induction and penetration of E7 specific CD8$^+$ T cells in the spleens and tumors of mice administered TC-1 cells and subsequently administered a recombinant *Listeria* vaccine (naive, Lm-LLO-E7, Lm-E7, Lm-ActA-E7).

FIG. **14**. A. *Listeria* constructs containing PEST regions lead to greater tumor regression. B. average tumor size in mice treated with Listeria vaccines.

FIG. **15**. *Listeria* constructs containing PEST regions induce a higher percentage of E7-specific lymphocytes in the spleen. A. data from 1 representative experiment. B. average and SE of data from 3 experiments.

FIG. **16**. *Listeria* constructs containing PEST regions induce a higher percentage of E7-specific lymphocytes within the tumor. A. data from 1 representative experiment. B. average and SE of data from 3 experiments.

FIG. **17**. Depiction of vaccinia virus constructs expressing different forms of HPV16E7 protein.

FIG. **18**. VacLLOE7 induces long-term regression of tumors established from 2×10^5 TC-1 cells in C57BL/6 mice. Mice were injected 11 and 18 days after tumor challenge with 10^7 PFU of VacLLOE7, VacSigE7LAMP-1, or VacE7/mouse i.p. or were left untreated (naive). 8 mice per treatment group were used, and the cross section for each tumor (average of 2 measurements) is shown for the indicated days after tumor inoculation.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides recombinant polypeptides comprising a fragment of a High Molecular Weight Melanoma-Associated Antigen (HMW-MAA), recombinant *Listeria* strains comprising same, and methods of inducing an immune response and treating and impeding the growth of tumors, comprising administering same.

In one embodiment, the present invention provides a recombinant *Listeria* strain comprising a recombinant polypeptide, the recombinant polypeptide comprising a fragment of a HMW-MAA protein ("HMW-MAA fragment"). In another embodiment, a recombinant *Listeria* strain of the present invention expresses a recombinant polypeptide of the present invention. In another embodiment, a recombinant *Listeria* strain of the present invention comprises an isolated nucleic acid that encodes a recombinant polypeptide of the present invention. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a recombinant polypeptide comprising a fragment of a HMW-MAA protein operatively linked to a non-HMW-MAA oligopeptide selected from a listeriolysin (LLO) oligopeptide,

an ActA oligopeptide, or a PEST-like oligopeptide or a fragment thereof. In one embodiment, the fragment has the same or a similar properties or function as the full length peptide or protein, as may be demonstrated using assays and tools known in the art. Properties and functions of full length peptides and proteins of the present invention are described in detail hereinbelow.

In another embodiment, the present invention provides a recombinant polypeptide comprising a fragment of a HMW-MAA protein, wherein the fragment consists of about amino acids (AA) 360-554 of the HMW-MAA protein from which the fragment is derived. In another embodiment, the fragment consists of about AA 701-1130. In another embodiment, the fragment has a sequence selected from SEQ ID No: 21-23. In another embodiment, the fragment consists of about AA 2160-2258. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a recombinant polypeptide comprising a fragment of a HMW-MAA protein with an amino acid sequence as set forth in SEQ ID No: 21.

In another embodiment, the present invention provides a recombinant polypeptide comprising a fragment of a HMW-MAA protein with an amino acid sequence as set forth in SEQ ID No: 22.

In another embodiment, the present invention provides a recombinant polypeptide comprising a fragment of a HMW-MAA protein with an amino acid sequence as set forth in SEQ ID No: 23.

In another embodiment, a recombinant polypeptide of the present invention further comprises a non-HMW-MAA peptide. In another embodiment, the non-HMW-MAA peptide enhances the immunogenicity of the fragment. Each possibility represents a separate embodiment of the present invention.

The non-HMW-MAA peptide is, in another embodiment, a listeriolysin (LLO) oligopeptide. In another embodiment, the non-HMW-MAA peptide is an ActA oligopeptide. In another embodiment, the non-HMW-MAA peptide is a PEST-like oligopeptide. As provided herein, fusion to LLO, ActA, PEST-like sequences and fragments thereof enhances the cell-mediated immunogenicity of antigens. In one embodiment, fusion to LLO, ActA, PEST-like sequences and fragments thereof enhances the cell-mediated immunogenicity of antigens in a variety of expression systems. In one embodiment, the expression system is viral, while in another embodiment, the expression system is bacterial. In another embodiment, the non-HMW-MAA peptide is any other immunogenic non-HMW-MAA peptide known in the art. Each possibility represents a separate embodiment of the present invention.

An LLO oligopeptide of methods and compositions of the present invention is, in another embodiment, a non-hemolytic LLO oligopeptide. In another embodiment, the oligopeptide is an LLO fragment. In another embodiment, the oligopeptide is a complete LLO protein. In another embodiment, the oligopeptide is any LLO protein or fragment thereof known in the art. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the LLO fragment is rendered non-hemolytic by chemical treatment. In another embodiment, the chemical treatment comprises glutaraldehyde. In another embodiment, the chemical treatment comprises a similarly acting compound. In another embodiment, the chemical treatment comprises any other suitable compound known in the art. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the LLO protein utilized to construct vaccines of the present invention has the following sequence:

MKKIMLVFITILVSLPIAQQTEAKDASA-
FNKENSISSMAPPASPPASPKTPIEKKHADEIDKYIQ
GLDYNKNNVLVYHGDAVTNVP-
PRKGYKDGNEYIVVEKKKKSINQN-
NADIQVVNAISSLTYPG ALVKANSELVENQPDV-
LPVKRDSLTLSIDLPGMTNQDNKIVVKNATKSNVNN-
AVNTLVERWN EKYAQAYPNVSAKIDYDDEMAY-
SESQLIAKFGTAFKAVNNSLNVNFGAI-
SEGKMQEEVISFKQ IYYNVNVNEPTRPSRFFGKAVT-
KEQLQALGVNAENPPAYISSVAYGRQVYLKLSTNSH-
STKVK AAFDAAVSGKSVSGDVELTNIIKNSS-
FKAVIYGGSAKDEVQIIDGNLGDLRDIL-
KKGATFNRETP GVPIAYTTNFLKDNELAVIKNNSEYI-
ETTSKAYTDGKINIDHSGGYVAQFNISWDEVNYDPE-
GN EIVQHKNWSENNKSKLAHFTSSIYLPG-
NARNINVYAKECTGLAWEWWRTVIDDRNLPLVKNR
NISIWGTTLYPKYSNKVDNPIE (GenBank Accession No. P13128; SEQ ID NO: 1; the nucleic acid sequence is set forth in GenBank Accession No. X15127). In one embodiment, the first 25 AA of the proprotein corresponding to this sequence are the signal sequence and are cleaved from LLO when it is secreted by the bacterium. Thus, according to this embodiment, the full length active LLO protein is 504 residues long. In another embodiment, the above sequence is used as the source of the LLO fragment incorporated in a vaccine of the present invention. In another embodiment, an LLO AA sequence of methods and compositions of the present invention is a homologue of SEQ ID No: 1. In another embodiment, the LLO AA sequence is a variant of SEQ ID No: 1. In another embodiment, the LLO AA sequence is a fragment of SEQ ID No: 1. In another embodiment, the LLO AA sequence is an isoform of SEQ ID No: 1. Each possibility represents a separate embodiment of the present invention.

In one embodiment, an isoform is a peptide or protein that has the same function and a similar or identical sequence to another peptide or protein, but is the product of a different gene. In one embodiment, a variant is a peptide or protein that differs from another a peptide or protein in a minor way.

In another embodiment, an LLO protein fragment is utilized in compositions and methods of the present invention. In another embodiment, the LLO fragment is an N-terminal fragment. In another embodiment, the N-terminal LLO fragment has the sequence:

MKKIMLVFITLILVSLPIAQQTEAK-
DASAFNKENSISSVAPPASPPASPKT-
PIEKKHADEIDKYIQ GLDYNKNNVLVYHGDAVT-
NVPPRKGYKDGNEYIVVEKKKKSINQNNADIQVVN-
AISSLTYP GALVKANSELVENQPDVLPVKRDSLTL-
SIDLPGMTNQDNKIVVKNATKSNVNNAVNTLVER
WNEKYAQAYSNVSAKIDYDDEMAY-
SESQLIAKFGTAFKAVNNSLNVNFGAI-
SEGKMQEEVIS FKQIYYNVNVNEPTRPSRFFGKAVT-
KEQLQALGVNAENPPAYISSVAYGRQVYLKLSTNSH-
ST KVKAAFDAAVSGKSVSGDVELTNIIKNS-
SFKAVIYGGSAKDEVQIIDGNLGDLRDLKKGATFN
RETPGVPIAYTTNFLKDNELA-
VIKNNSEYIETTSKAYTDGKINIDHSG-
GYVAQFNISWDEVNYD (SEQ ID NO: 2). In another embodiment, an LLO AA sequence of methods and compositions of the present invention comprises the sequence set forth in SEQ ID No: 2. In another embodiment, the LLO AA sequence is a homologue of SEQ ID No: 2. In another embodiment, the LLO AA sequence is a variant of SEQ ID No: 2. In another embodiment, the LLO AA sequence is a

fragment of SEQ ID No: 2. In another embodiment, the LLO AA sequence is an isoform of SEQ ID No: 2. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the LLO fragment has the sequence:

MKKIMLVFITLILVSLPIAQQTEAK-
DASAFNKENSISSVAPPASPPASPKT-
PIEKKHADEIDKYIQ GLDYNKNNVLVYHGDAVT-
NVPPRKGYKDGNEYIVVEKKKKSINQNNADIQVVN-
AISSLTYP GALVKANSELVENQPDVLPVKRDSLTL-
SIDLPGMTNQDNKIVVKNATKSNVNNAVNTLVER
WNEKYAQAYSNVSAKIDYDDEMAY-
SESQLIAKFGTAFKAVNNSLNVNFGAISEGKMQEEVI
SFKQIYYNVNVNEPTRPSRFFGKAVT-
KEQLQALGVNAENPPAYISSVAYGRQVYLKLSTNSH
STKVKAAFDAAVSGKSVSGDVELTNI-
IKNSSFKAVIYGGSAKDEVQIIDGNLGDLRDILKKGA
TFNRETPGVPIAYTTNFLKDNELA-
VIKNNSEYIETTSKAYTD (SEQ ID NO: 3). In another embodiment, an LLO AA sequence of methods and compositions of the present invention comprises the sequence set forth in SEQ ID No: 3. In another embodiment, the LLO AA sequence is a homologue of SEQ ID No: 3. In another embodiment, the LLO AA sequence is a variant of SEQ ID No: 3. In another embodiment, the LLO AA sequence is a fragment of SEQ ID No: 3. In another embodiment, the LLO AA sequence is an isoform of SEQ ID No: 3. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the LLO fragment of methods and compositions of the present invention comprises a PEST-like domain. In another embodiment, an LLO fragment that comprises a PEST sequence is utilized as part of a composition or in the methods of the present invention.

In another embodiment, the LLO fragment does not contain the activation domain at the carboxy terminus. In another embodiment, the LLO fragment does not include cysteine 484. In another embodiment, the LLO fragment is a non-hemolytic fragment. In another embodiment, the LLO fragment is rendered non-hemolytic by deletion or mutation of the activation domain. In another embodiment, the LLO fragment is rendered non-hemolytic by deletion or mutation of cysteine 484. In another embodiment, an LLO sequence is rendered non-hemolytic by deletion or mutation at another location.

In another embodiment, the LLO fragment consists of about the first 441 AA of the LLO protein. In another embodiment, the LLO fragment comprises about the first 400-441 AA of the 529 AA full length LLO protein. In another embodiment, the LLO fragment corresponds to AA 1-441 of an LLO protein disclosed herein. In another embodiment, the LLO fragment consists of about the first 420 AA of LLO. In another embodiment, the LLO fragment corresponds to AA 1-420 of an LLO protein disclosed herein. In another embodiment, the LLO fragment consists of about AA 20-442 of LLO. In another embodiment, the LLO fragment corresponds to AA 20-442 of an LLO protein disclosed herein. In another embodiment, any ΔLLO without the activation domain comprising cysteine 484, and in particular without cysteine 484, are suitable for methods and compositions of the present invention.

In another embodiment, the LLO fragment corresponds to the first 400 AA of an LLO protein. In another embodiment, the LLO fragment corresponds to the first 300 AA of an LLO protein. In another embodiment, the LLO fragment corresponds to the first 200 AA of an LLO protein. In another embodiment, the LLO fragment corresponds to the first 100 AA of an LLO protein. In another embodiment, the LLO fragment corresponds to the first 50 AA of an LLO protein, which in one embodiment, comprises one or more PEST-like sequences.

In another embodiment, the LLO fragment contains residues of a homologous LLO protein that correspond to one of the above AA ranges. The residue numbers need not, in another embodiment, correspond exactly with the residue numbers enumerated above; e.g. if the homologous LLO protein has an insertion or deletion, relative to an LLO protein utilized herein.

Each LLO protein and LLO fragment represents a separate embodiment of the present invention.

In another embodiment, homologues of LLO from other species, including known lysins, or fragments thereof may be used as the non-HMW-MAA.

In another embodiment of methods and compositions of the present invention, a fragment of an ActA protein is fused to the HMW-MAA fragment. In another embodiment, the fragment of an ActA protein has the sequence:

MRAMMVVFITANCITINPDIIFAATD-
SEDSSLNTDEWEEEKTEEQPSEVNTGPRYETAR
EVSSRDIKELEKSNKVRNTNKADLIAM-
LKEKAEKGPNINNNNSEQTENAAINEEASGADRPAI
QVERRHPGLPSDSAAEIKKRRKAIASSD-
SELESLTYPDKPTKVNKKKVAKESVADASESDLDS
SMQSADESSPQPLKANQQPFFPKVFK-
KIKDAGKWVRDKIDENPEVKKAIVDKSA-
GLIDQLLTK KKSEEVNASDFPPPPTDEELRLALPETP-
MLLGFNAPATSEPSSFEFPPPPTDEELRLALPETPMLL
GFNAPATSEPSSFEFPPPPTEDELEI-
IRETASSLDSSFTRGDLASLR-
NAINRHSQNFSDFPPIPTEEE LNGRGGRP (SEQ ID No: 4). In another embodiment, an ActA AA sequence of methods and compositions of the present invention comprises the sequence set forth in SEQ ID No: 4. In another embodiment, the ActA AA sequence is a homologue of SEQ ID No: 4. In another embodiment, the ActA AA sequence is a variant of SEQ ID No: 4. In another embodiment, the ActA AA sequence is a fragment of SEQ ID No: 4. In another embodiment, the ActA AA sequence is an isoform of SEQ ID No: 4. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the ActA fragment is encoded by a recombinant nucleotide comprising the sequence:

ATGCGTGCGATGATGGTGGTTTTCAT-
TACTGCCAATTGCATTACGATTAACCCCGACATAA
TATTTGCAGCGACAGATAGCGAAGAT-
TCTAGTCTAAACACAGATGAATGGGAAGAAGAAA
AAACAGAAGAGCAACCAAGCGAGG-
TAAATACGGGACCAAGATACGAAACTGCACGTGAA
GTAAGTTCACGTGATATTAAAGAACTA-
GAAAAATCGAATAAAGTGAGAAATACGAACAAA
GCAGACCTAATAGCAATGTTGAAA-
GAAAAAGCAGAAAAAGGTCCAAATAT-
CAATAATAAC AACAGTGAACAAACTGAGAATGCG-
GCTATAAATGAAGAGGCTTCAGGAGCCGACCGACC-
A GCTATACAAGTGGAGCGTCGTCATCCAG-
GATTGCCATCGGATAGCGCAGCGGAAATTAAAA
AAAGAAGGAAAGCCATAGCATCATCG-
GATAGTGAGCTTGAAAGCCTTACTTATCCGGATAA
ACCAACAAAAGTAAATAAGAAAAAAGTG-
GCGAAAGAGTCAGTTGCGGATGCTTCTGAAAG
TGACTTAGATTCTAGCATGCAGTCAGCA-
GATGAGTCTTCACCACAACCTTTAAAAGCAAAC
CAACAACCATTTTTCCCTAAAGTATT-
TAAAAAAATAAAAGATGCGGGGAAATGGGTACGTG
ATAAAATCGACGAAAATCCTGAAGTAAA-

GAAAGCGATTGTTGATAAAAGTGCAGGGTTAA
TTGACCAATTATTAACCAAAAA-
GAAAAGTGAAGAGGTAAATGCTTCG-
GACTTCCCGCCACC ACCTACGGATGAAGAGTTAA-
GACTTGCTTTGCCAGAGACACCAATGCTTCTTGGT-
TTTAAT GCTCCTGCTACATCAGAACCGAGCTCAT-
TCGAATTTCCACCACCACCTACGGATGAAGAGT
TAAGACTTGCTTTGCCAGAGACGCCAAT-
GCTTCTTGGTTTTAATGCTCCTGCTACATCGGAA
CCGAGCTCGTTCGAATTTCCACCGCCTC-
CAACAGAAGATGAACTAGAAATCATCCGGGAA
ACAGCATCCTCGCTAGATTCTAGTTTTA-
CAAGAGGGGATTTAGCTAGTTTGAGAAATGCTA
TTAATCGCCATAGTCAAAATTTCTCT-
GATTTCCCACCAATCCCAACAGAAGAAGAGTTGAA
CGGGAGAGGCGGTAGACCA (SEQ ID NO: 5). In another embodiment, the recombinant nucleotide has the sequence set forth in SEQ ID NO: 5. In another embodiment, an ActA-encoding nucleotide of methods and compositions of the present invention comprises the sequence set forth in SEQ ID No: 5. In another embodiment, the ActA-encoding nucleotide is a homologue of SEQ ID No: 5. In another embodiment, the ActA-encoding nucleotide is a variant of SEQ ID No: 5. In another embodiment, the ActA-encoding nucleotide is a fragment of SEQ ID No: 5. In another embodiment, the ActA-encoding nucleotide is an isoform of SEQ ID No: 5. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the ActA fragment is any other ActA fragment known in the art. In another embodiment, a recombinant nucleotide of the present invention comprises any other sequence that encodes a fragment of an ActA protein. In another embodiment, the recombinant nucleotide comprises any other sequence that encodes an entire ActA protein. Each possibility represents a separate embodiment of the present invention.

In another embodiment of methods and compositions of the present invention, a PEST-like AA sequence is fused to the HMW-MAA fragment. In another embodiment, the PEST-like AA sequence is KENSISSMAPPASPPASPKT-PIEKKHADEIDK (SEQ ID NO: 6). In another embodiment, the PEST-like sequence is KENSISSMAPPASPPASPK (SEQ ID No: 7). In another embodiment, fusion of an antigen to any LLO sequence that includes the 1 of the PEST-like AA sequences enumerated herein can enhance cell mediated immunity against HMW-MAA.

In another embodiment, the PEST-like AA sequence is a PEST-like sequence from a *Listeria* ActA protein. In another embodiment, the PEST-like sequence is KTEEQPSEVNT-GPR (SEQ ID NO: 8), KASVTDTSEGDLDSSM-QSADESTPQPLK (SEQ ID NO: 9), KNEEVNASDFPP-PPTDEELR (SEQ ID NO: 10), or RGGIPTSEEFSSLNSGDFTDDENSETTEEEIDR (SEQ ID NO: 11). In another embodiment, the PEST-like sequence is from *Listeria seeligeri* cytolysin, encoded by the lso gene. In another embodiment, the PEST-like sequence is RSEVTIS-PAETPESPPATP (SEQ ID NO: 12). In another embodiment, the PEST-like sequence is from Streptolysin O protein of *Streptococcus* sp. In another embodiment, the PEST-like sequence is from *Streptococcus pyogenes* Streptolysin O, e.g. KQNTASTETTTTNEQPK (SEQ ID NO: 13) at AA 35-51. In another embodiment, the PEST-like sequence is from *Streptococcus equisimilis* Streptolysin O, e.g. KQNTAN-TETTTTNEQPK (SEQ ID NO: 14) at AA 38-54. In another embodiment, the PEST-like sequence has a sequence selected from SEQ ID NO: 8-14. In another embodiment, the PEST-like sequence has a sequence selected from SEQ ID NO: 6-14. In another embodiment, the PEST-like sequence is another PEST-like AA sequence derived from a prokaryotic organism.

Identification of PEST-like sequences is well known in the art, and is described, for example in Rogers S et al (Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. Science 1986; 234(4774):364-8) and Rechsteiner M et al (PEST sequences and regulation by proteolysis. Trends Biochem Sci 1996; 21(7):267-71). "PEST-like sequence" refers, in another embodiment, to a region rich in proline (P), glutamic acid (E), serine (S), and threonine (T) residues. In another embodiment, the PEST-like sequence is flanked by one or more clusters containing several positively charged amino acids. In another embodiment, the PEST-like sequence mediates rapid intracellular degradation of proteins containing it. In another embodiment, the PEST-like sequence fits an algorithm disclosed in Rogers et al. In another embodiment, the PEST-like sequence fits an algorithm disclosed in Rechsteiner et al. In another embodiment, the PEST-like sequence contains one or more internal phosphorylation sites, and phosphorylation at these sites precedes protein degradation.

In one embodiment, PEST-like sequences of prokaryotic organisms are identified in accordance with methods such as described by, for example Rechsteiner and Rogers (1996, Trends Biochem. Sci. 21:267-271) for LM and in Rogers S et al (Science 1986; 234(4774):364-8). Alternatively, PEST-like AA sequences from other prokaryotic organisms can also be identified based on this method. Other prokaryotic organisms wherein PEST-like AA sequences would be expected to include, but are not limited to, other Listeria species. In one embodiment, the PEST-like sequence fits an algorithm disclosed in Rogers et al. In another embodiment, the PEST-like sequence fits an algorithm disclosed in Rechsteiner et al. In another embodiment, the PEST-like sequence is identified using the PEST-find program.

In another embodiment, identification of PEST motifs is achieved by an initial scan for positively charged AA R, H, and K within the specified protein sequence. All AA between the positively charged flanks are counted and only those motifs are considered further, which contain a number of AA equal to or higher than the window-size parameter. In another embodiment, a PEST-like sequence must contain at least 1 P, 1 D or E, and at least 1 S or T.

In another embodiment, the quality of a PEST motif is refined by means of a scoring parameter based on the local enrichment of critical AA as well as the motif's hydrophobicity. Enrichment of D, E, P, S and T is expressed in mass percent (w/w) and corrected for 1 equivalent of D or E, 1 of P and 1 of S or T. In another embodiment, calculation of hydrophobicity follows in principle the method of J. Kyte and R. F. Doolittle (Kyte, J and Dootlittle, R F. J. Mol. Biol. 157,105 (1982). For simplified calculations, Kyte-Doolittle hydropathy indices, which originally ranged from −4.5 for arginine to +4.5 for isoleucine, are converted to positive integers, using the following linear transformation, which yielded values from 0 for arginine to 90 for isoleucine.

Hydropathy index=10*Kyte-Doolittle hydropathy index+45

In another embodiment, a potential PEST motif's hydrophobicity is calculated as the sum over the products of mole percent and hydrophobicity index for each AA species. The desired PEST score is obtained as combination of local enrichment term and hydrophobicity term as expressed by the following equation:

PEST score=0.55**DEPST*−0.5*hydrophobicity index.

In another embodiment, "PEST-like sequence" or "PEST-like sequence peptide" refers to a peptide having a score of at least +5, using the above algorithm. In another embodiment, the term refers to a peptide having a score of at least 6. In another embodiment, the peptide has a score of at least 7. In another embodiment, the score is at least 8. In another embodiment, the score is at least 9. In another embodiment, the score is at least 10. In another embodiment, the score is at least 11. In another embodiment, the score is at least 12. In another embodiment, the score is at least 13. In another embodiment, the score is at least 14. In another embodiment, the score is at least 15. In another embodiment, the score is at least 16. In another embodiment, the score is at least 17. In another embodiment, the score is at least 18. In another embodiment, the score is at least 19. In another embodiment, the score is at least 20. In another embodiment, the score is at least 21. In another embodiment, the score is at least 22. In another embodiment, the score is at least 22. In another embodiment, the score is at least 24. In another embodiment, the score is at least 24. In another embodiment, the score is at least 25. In another embodiment, the score is at least 26. In another embodiment, the score is at least 27. In another embodiment, the score is at least 28. In another embodiment, the score is at least 29. In another embodiment, the score is at least 30. In another embodiment, the score is at least 32. In another embodiment, the score is at least 35. In another embodiment, the score is at least 38. In another embodiment, the score is at least 40. In another embodiment, the score is at least 45. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the PEST-like sequence is identified using any other method or algorithm known in the art, e.g the CaSPredictor (Garay-Malpartida H M, Occhiucci J M, Alves J, Belizario J E. Bioinformatics. 2005 June; 21 Suppl 1:i169-76). In another embodiment, the following method is used:

A PEST index is calculated for each stretch of appropriate length (e.g. a 30-35 AA stretch) by assigning a value of 1 to the AA Ser, Thr, Pro, Glu, Asp, Asn, or Gln. The coefficient value (CV) for each of the PEST residue is 1 and for each of the other AA (non-PEST) is 0.

Each method for identifying a PEST-like sequence represents a separate embodiment of the present invention.

In another embodiment, the PEST-like sequence is any other PEST-like sequence known in the art. Each PEST-like sequence and type thereof represents a separate embodiment of the present invention.

"Fusion to a PEST-like sequence" refers, in another embodiment, to fusion to a protein fragment comprising a PEST-like sequence. In another embodiment, the term includes cases wherein the protein fragment comprises surrounding sequence other than the PEST-like sequence. In another embodiment, the protein fragment consists of the PEST-like sequence. Thus, in another embodiment, "fusion" refers to two peptides or protein fragments either linked together at their respective ends or embedded one within the other. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the HMW-MAA fragment of methods and compositions of the present invention is fused to the non-HMW-MAA AA sequence. In another embodiment, the HMW-MAA fragment is embedded within the non-HMW-MAA AA sequence. In another embodiment, an HMW-MAA-derived peptide is incorporated into an LLO fragment, ActA protein or fragment, or PEST-like sequence. Each possibility represents a separate embodiment of the present invention.

In another embodiment, fusion proteins of the present invention are prepared by a process comprising subcloning of appropriate sequences, followed by expression of the resulting nucleotide. In another embodiment, subsequences are cloned and the appropriate subsequences cleaved using appropriate restriction enzymes. The fragments are then ligated, in another embodiment, to produce the desired DNA sequence. In another embodiment, DNA encoding the fusion protein is produced using DNA amplification methods, for example polymerase chain reaction (PCR). First, the segments of the native DNA on either side of the new terminus are amplified separately. The 5' end of the one amplified sequence encodes the peptide linker, while the 3' end of the other amplified sequence also encodes the peptide linker. Since the 5' end of the first fragment is complementary to the 3' end of the second fragment, the two fragments (after partial purification, e.g. on LMP agarose) can be used as an overlapping template in a third PCR reaction. The amplified sequence will contain codons, the segment on the carboxy side of the opening site (now forming the amino sequence), the linker, and the sequence on the amino side of the opening site (now forming the carboxyl sequence). The insert is then ligated into a plasmid. In another embodiment, a similar strategy is used to produce a protein wherein an HMW-MAA fragment is embedded within a heterologous peptide.

In one embodiment, LLO sequences fused to a HMW-MAA fragment such as A, B, or C or Listeria expressing a HMW-MAA fragment increased the immune response to said peptide (Example 5), conferred antitumor immunity (Examples 4 and 5), and generated peptide-specific IFN-gamma-secreting CD8+ cells (Example 5). In one embodiment, ActA, LLO and/or PEST-like sequences fused to a peptide such as HPV E7 increased the immune response to said peptide, conferred antitumor immunity, and generated peptide-specific IFN-gamma-secreting CD8+ cells (Examples 6 and 7), even when the fusion peptide was expressed in a non-Listeria vector (Example 8).

In another embodiment, a recombinant polypeptide of the present invention is made by a process comprising the step of chemically conjugating a first polypeptide comprising an HMW-MAA fragment to a second polypeptide comprising a non-HMW-MAA peptide. In another embodiment, an HMW-MAA fragment is conjugated to a second polypeptide comprising the non-HMW-MAA peptide. In another embodiment, a peptide comprising an HMW-MAA fragment is conjugated to a non-HMW-MAA peptide. In another embodiment, an HMW-MAA fragment is conjugated to a non-HMW-MAA peptide. Each possibility represents a separate embodiment of the present invention.

The HMW-MAA protein from which HMW-MAA fragments of the present invention are derived is, in another embodiment, a human HMW-MAA protein. In another embodiment, the HMW-MAA protein is a mouse protein. In another embodiment, the HMW-MAA protein is a rat protein. In another embodiment, the HMW-MAA protein is a primate protein. In another embodiment, the HMW-MAA protein is from any other species known in the art. In another embodiment, the HMW-MAA protein is melanoma chondroitin sulfate proteoglycan (MCSP). In another embodiment, an AN2 protein is used in methods and compositions of the present invention. In another embodiment, an NG2 protein is used in methods and compositions of the present invention.

In another embodiment, the HMW-MAA protein of methods and compositions of the present invention has the sequence:

MQSGRGPPLPAPGLALALTLTMLARLA-
SAASFFGENHLEVPVATALTDIDLQLQFSTSQ

PEALLLLAAGPADHLLLQLYSGRLQVR-
LVLGQEELRLQTPAETLLSDSIPHTVVLTVVEGWATL
SVDGFLNASSAVPGAPLEVPYGLFVGGT-
GTLGLPYLRGTSRPLRGCLHAATLNGRSLLRPLTPD
VHEGCAEEFSASDDVALGFSGPHSLAAF-
PAWGTQDEGTLEFTLTTQSRQAPLAFQAGGRRGDF
IYVDIFEGHLRAVVEKGQGTVLLHNSVP-
VADGQPHEVSVHINAHRLEISVDQYPTHTSNRGVLS
YLEPRGSLLLGGLDAEASRHLQEHR-
LGLTPEATNASLLGCMEDLSVNGQRRGL-
REALLTRNMA AGCRLEEEEYEDDAYGHYEAFSTLA-
PEAWPAMELPEPCVPEPGLPPVFANFTQLLTISPLVVAE
GGTAWLEWRHVQPTLDLMEAELRKSQV-
LFSVTRGARHGELELDIPGAQARKMFTLLDVVNR
KARFIHDGSEDTSDQLVLEVSVTARVP-
MPSCLRRGQTYLLPIQVNPVNDPPHIIFPHGSLMVILE
HTQKPLGPEVFQAYDPDSACEGLTFQV-
LGTSSGLPVERRDQPGEPATEFSCRELEAGSLVYVH
RGGPAQDLTFRVSDGLQASPPATLKV-
VAIRPAIQIHRSTGLRLAQGSAMPILPANLSVETNAVG
QDVSVLFRVTGALQFGELQKQGAGGVEG-
AEWWATQAFHQRDVEQGRVRYLSTDPQHHAYD
TVENLALEVQVGQEILSNLSFPV-
TIQRATVWMLRLEPLHTQNTQQETLT-
TAHLEATLEEAGPSPP TFHYEVVQAPRKGN-
LQLQGTRLSDGQGFTQDDIQAGRVTYGATARASEAV-
EDTFRFRVTAPPY FSPLYTFPIHIGGDPDAPVLTNV-
LLVVPEGGEGVLSADHLFVKSLNSASY-
LYEVMERPRHGRLA WRGTQDKTTMVTSFTNEDLL-
RGRLVYQHDDSETTEDDIPFVATRQGESSGDMAWEE-
VRGVFR VAIQPVNDHAPVQTISRIFHVARGGR-
RLLTTDDVAFSDADSGFADAQLVL-
TRKDLLFGSIVAVD EPTRPIYRFTQEDLRKRRVLFVH-
SGADRGWIQLQVSDGQHQATALLEVQASEPYLRVA-
NGSSL VVPQGGQGTIDTAVLHLDTNLDIRS-
GDEVHYHVTAGPRWGQLVRAGQ-
PATAFSQQDLLDGAV LYSHNGSLSPRDTMAFS-
VEAGPVHTDATLQVTIALEGPLAPLKLVRHKKIYVF-
QGEAAEIRRDQ LEAAQEAVPPADIVFSVKSPP-
SAGYLVMVSRGALADEPPSLDPVQSF-
SQEAVDTGRVLYLHSRP EAWSDAFSLDVASGL-
GAPLEGVLVELEVLPAAIPLEAQNFSVPEGGSLTLAP-
PLLRVSGPYFPTL LGLSLQVLEPPQHGALQKEDG-
PQARTLSAFSWRMVEEQLIRYVHDG-
SETLTDSFVLMANASEM DRQSHPVAFTVTV-
LPVNDQPPILTTNTGLQMWEGATAPIPAEALRSTDGD-
SGSEDLVYTIEQPS NGRVVLRGAPGTEVRS-
FTQAQLDGGLVLFSHRGTLDGGFRFRLS-
DGEHTSPGHFFRVTAQKQV LLSLKGSQTLTVCPGS-
VQPLSSSQTLRASSSAGTDPQLLLYRVVRGPQLGRLF-
HAQQDSTGEALV NFTQAEVYAGNILYEHEMPPEPF-
WEAHDTLELQLSSPPARDVAATLAVAVS-
FEAACPQRPSHL WKNKGLWVPEGQRARITVAAL-
DASNLLASVPSPQRSEHDVLFQVTQFPSRGQLLVSEE-
PLHAG QPHFLQSQLAAGQLVYAHGGGGTQQDGF-
HFRAHLQGPAGASVAGPQTSEAFAITVRDVNERP
PQPQASVPLRLTRGSRAPISRAQLSVVD-
PDSAPGEIEYEVQRAPHNGFLSLVGGGLGPVTRFTQA
DVDSGRLAFVANGSSVAGIFQLSMSD-
GASPPLPMSLAVDILPSAIEVQLRA-
PLEVPQALGRSSLS QQQLRVVS DREEPEAAYRLIQG-
PQYGHLLVGGRPTSAFSQFQIDQGEVVFAFTNFSSSH-
DHFRV LALARGVNASAVVNVTVRALLHVWAGGP-
WPQGATLRLDPTVLDAGELANRTGSVPRFRLLE
GPRHGRV-
VRVPRARTEPGGSQLVEQFTQQDLEDGR-
LGLEVGRPEGRAPGPAGDSLTLELWAQ GVP-
PAVASLDFATEPYNAARPYSVALLSVPEAARTEAGKP-
ESSTPTGEPGPMASSPEPAVAKG GFLSFLEANMFSVI-
IPMCLVLLLLALILPLLFYLRKRNKT-
GKHDVQVLTAKPRNGLAGDTETFR KVEPGQAIPL-
TAVPGQGPPPGGQPDPELLQFCRTPNPALKNGQYWV
(SEQ ID No: 15). In another embodiment, an HMW-MAA AA sequence of methods and compositions of the present invention comprises the sequence set forth in SEQ ID No: 15. In another embodiment, the HMW-MAA AA sequence is a homologue of SEQ ID No: 15. In another embodiment, the HMW-MAA AA sequence is a variant of SEQ ID No: 15. In another embodiment, the HMW-MAA AA sequence is a fragment of SEQ ID No: 15. In another embodiment, the HMW-MAA AA sequence is an isoform of SEQ ID No: 15. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the HMW-MAA protein of methods and compositions of the present invention is encoded by the sequence:

atgcagtccggccgcggcccccacttc-
cagcccccggcctggccttggctttgac-
cctgactatgttggccagacttgcatccgcggcttccttcttcg gtgagaaccac-
ctggaggtgcctgtggccacggctctgaccgacatagacctgcagctgcagttctc-
cacgtcccagcccgaagccctccttctcctg gcagcaggcccagctgaccac-
ctcctgctgcagctctactctggacgc-
ctgcaggtcagacttgttctgggccaggaggagctgaggctgcagactc cagca-
gagacgctgctgagtgactccatcccccacactgtggtgctgactgtcgtagagg-
gctgggccacgttgtcagtcgatgggtttctgaacgcct cctcagcagtcccag-
gagcccccctagaggtc-
ccctatgggctctttgt-
tgggggcactgggacccttggcctgccctacctgaggggaaccagccg
acccctgaggggttgcctccatgcagc-
caccctcaatggccgcagcctcctccg-
gcctctgacccccgatgtgcatgagggctgtgctgaagagttttc tgccagtgat-
gatgtggccctgggcttctctgggcccccactctctggctgccttccctgcctgggg-
cactcaggacgaaggaaccctagagtttacactc accacacagagccggcag-
gcacccttggccttccaggcagggggc-
cggcgtggggacttcatctatgtggacatatttgagggccacctgcgggcc gtg-
gtggagaagggccagggtaccgtattgctccacaacagtgtgcctgtggccgatg-
ggcagccccatgaggtcagtgtccacatcaatgctcaccg gctggaaatctc-
cgtggaccagtaccctacgcatacttc-
gaaccgaggagtcctcagctacctg-
gagccacggggcagtctccttctcgggggggctgg
atgcagaggcctctcgtcacctccag-
gaacacccgcctgggcctgacaccagag-
gccaccaatgcctccctgctgggctgcatggaagacctcagtgtc aatggcca-
gaggcgggggctgcgggaagctttgctgacgcgcaacatggcagccggctgca-
ggctggaggaggaggagtatgaggacgatgccta tggacattatgaagctttctc-
caccctggcccctgaggcttggccagc-
catggagctgcctgagccatgcgtgcctgagccagggctgcctcctgtcttt
gccaatttcacccagctgctgactat-
cagcccactggtggtggc-
cgaggggggcacagcctggcttgagtggaggcatgtgcagcccacgctggacct
gatggaggctgagctgcgcaaatcccag-
gtgctgttcagcgtgac-
ccgaggggcacgccatggcgagctcgagctggacatcccgggagcccagg
cacgaaaaatgttcaccctcctg-
gacgtggtgaaccgcaaggcccgct-
tcatccacgatggctctgaggacacctccgaccagctggtgctggaggtg tcggt-
gacggctcgggtgcccatgccctcatgccttcggaggggccaaacatacctcctg-
cccatccaggtcaaccctgtcaatgacccaccccacatc atcttcccacatg-
gcagcctcatggtgatcctggaaca-
cacgcagaagccgctggggcctgag-
gttttccaggcctatgacccggactctgcctgtgag
ggcctcaccttccaggtccttggcac-
ctcctctggcctccccgtggagcgccgagaccag-
cctggggagccggcgaccgagttctcctgccgggagtt ggaggccggcagc-

ctagtctatgtccaccgcggtggtcctg-
cacaggacttgacgttccgggtcagc-
gatggactgcaggccagccccccggccac
gctgaaggtggtggccatccggccggc-
catacagatccaccgcagcacagggt-
tgcgactggcccaaggctctgccatgcccatcttgcccgccaac ctgtcggtg-
gagaccaatgccgtggggcaggatgtgagcgtgctgttccgcgtcactggggcc-
ctgcagtttggggagctgcagaagcaggggcag gtggggtggagggtgct-
gagtggtgggccacacaggcgttccac-
cagcgggatgtggagcagggccgcgtgaggtacctgagcactgacccacagc
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ctggccctggaggtgcaggtgggccag-
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cagcccacctggaggccaccctggaggaggc aggcccaagccccccaacct-
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tggtggtgacccagatgcgcctgtcct-
caccaatgtcctcctcgtggtgcctgagggtgg
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ctctttgtcaagagtctcaacagtgc-
cagctacctctatgaggtcatggagcggcccgccatgggaggttggct
tggcgtgggacacaggacaagaccac-
tatggtgacatccttcaccaatgaagac-
ctgttgcgtggccggctggtctaccagcatgatgactccgagac cacagaagat-
gatatcccatttgttgctacccgccagggcgagagcagtggtgacatggcctggg-
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cctgtgcagaccatcagccggatcttc-
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gatgctgactcgggctttgctgacgcccagctggtgcttacccgcaaggacctcct-
ctttggcagtatcgtggccgtagatgagcccacgcggcc catctaccgcttcac-
ccaggaggacctcaggaagaggcgag-
tactgttcgtgcactcaggggctgac-
cgtggctggatccagctgcaggtgtccgacg
ggcaacaccaggccactgcgctgctg-
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gacctgctggatggggccgttctctatagccacaatggcagcctcagcc cccgc-
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gagcccaccgagtgccggctacctggt-
gatggtgtcgcgtggcgccttggcagatgagccacccagcctggac cctgtgca-
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gctcccctcgagggcgtccttgtg-
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agggtggcagcctcaccctggcccctc-
cactgctccgtgtctccgggccctact-
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cactgacagacagttttgtcctgatg-
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cgagtgacggcccagaagcaagtgctc-
ctctcgctgaagggcagccagacactgactgtctgcccagggtc cgtccagc-
cactcagcagtcagaccctcagggccagctccagcgcaggcactgaccccag-
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cagcgttccatcaccccagcgctcagag-
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gggcagctagtgtatgcccacggcggtgggg gcacccagcaggatggcttc-
cactttcgtgcccacctccaggggccag-
cagggcctccgtggctggaccccaaacctcagaggcctttgccatcac ggt-
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tcgtggccaacgggagcagcgtggcaggcatcttccagctgagcat gtct-
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gatcgggaggagccagaggcagcataccgcctcatccagggacccca
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gcccacctcggccttcagccaattcca-
gatagaccagggcgaggtggtctttgccttcaccaacttctcctcc tctcatgac-
cacttcagagtcctggcactggctagggtgtcaatgcatcagccgtagtgaacgt-
cactgtgagggctctgctgcatgtgtgggcaggtg ggccatggccccagggtgc-
cacctgcgcctggacccccaccgtccta-
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gagggaccccggcatggccgcgtggtccgcgtgccccgagccaggacggagc-
ccgggggcagccagctggtggagcagttcactcagca ggaccttgaggacgg-
gaggctggggctggaggtgggcaggcca-
gaggggagggccccccggccccgcaggtgacagtctcactctggagctgtgg
gcacagggcgtcccgcctgctgtggc-
ctccctggactttgccactgagcctta-
caatgctgcccggccctacagcgtggccctgctcagtgtccccgag gccgc-
ccggacggaagcagggaagccagagagcagcacccccacaggcgagccaggc-
ccccatggcatccagccctgagcccgctgtggccaag ggaggcttcctgagct-
tccttgaggccaacatgttcagcgtcat-
catccccatgtgcctggtacttctgctc-
ctggcgctcatcctgcccctgctcttctacct
ccgaaaacgcaacaagacgggcaagcat-
gacgtccaggtcctgactgccaagc-
cccgcaacggcctggctggtgacaccgagacctttcgcaaggt ggagccaggc-
caggccatcccgctcacagctgtgcctggccaggggccccctccaggaggcca-
gcctgacccagagctgctgcagttctgccgga cacccaaccctgcccttaa-
gaatggccagtactgggtgtgaggcctg-
gcctgggcccagatgctgatcgggccagggacaggc (SEQ ID No: 16).

In another embodiment, the recombinant nucleotide has the sequence set forth in SEQ ID NO: 16. In another embodiment, an HMW-MAA-encoding nucleotide of methods and compositions of the present invention comprises the sequence set forth in SEQ ID No: 16. In another embodiment, the HMW-MAA-encoding nucleotide is a homologue of SEQ ID No: 16.

In another embodiment, the HMW-MAA-encoding nucleotide is a variant of SEQ ID No: 16. In another embodiment, the HMW-MAA-encoding nucleotide is a fragment of SEQ ID No: 16. In another embodiment, the HMW-MAA-encoding nucleotide is an isoform of SEQ ID No: 16. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the HMW-MAA protein of methods and compositions of the present invention has an AA sequence set forth in a GenBank entry having an Accession Numbers selected from NM_001897 and X96753. In another embodiment, the HMW-MAA protein is encoded by a nucleotide sequence set forth in one of the above GenBank entries. In another embodiment, the HMW-MAA protein comprises a sequence set forth in one of the above GenBank entries. In another embodiment, the HMW-MAA protein is a homologue of a sequence set forth in one of the above GenBank entries. In another embodiment, the HMW-MAA protein is a variant of a sequence set forth in one of the above GenBank entries. In another embodiment, the HMW-MAA protein is a fragment of a sequence set forth in one of the above GenBank entries. In another embodiment, the HMW-MAA protein is an isoform of a sequence set forth in one of the above GenBank entries. Each possibility represents a separate embodiment of the present invention.

The HMW-MAA fragment utilized in the present invention comprises, in another embodiment, AA 360-554. In another embodiment, the fragment consists essentially of AA 360-554. In another embodiment, the fragment consists of AA 360-554. In another embodiment, the fragment comprises AA 701-1130. In another embodiment, the fragment consists essentially of AA 701-1130. In another embodiment, the fragment consists of AA 701-1130. In another embodiment, the fragment comprises AA 2160-2258. In another embodiment, the fragment consists essentially of 2160-2258. In another embodiment, the fragment consists of 2160-2258. Each possibility represents a separate embodiment of the present invention.

In some embodiments, a polypeptide of the present invention will comprise a fragment of a HMW-MAA protein, in any form or embodiment as described herein. In some embodiments, any of the polypeptides of the present invention will consist of a fragment of a HMW-MAA protein, in any form or embodiment as described herein. In some embodiments, of the compositions of this invention will consist essentially of a fragment of a HMW-MAA protein, in any form or embodiment as described herein. In some embodiments, the term "comprise" refers to the inclusion of the indicated active agent, such as the fragment of a HMW-MAA protein, or the fragment of a HMW-MAA protein and a non-HMW-MAA polypeptide, as well as inclusion of other active agents, and pharmaceutically acceptable carriers, excipients, emollients, stabilizers, etc., as are known in the pharmaceutical industry. In some embodiments, the term "consisting essentially of" refers to a composition, whose only active ingredient is the indicated active ingredient, however, other compounds may be included which are for stabilizing, preserving, etc. the formulation, but are not involved directly in the therapeutic effect of the indicated active ingredient. In some embodiments, the term "consisting essentially of" may refer to components which facilitate the release of the active ingredient. In some embodiments, the term "consisting" refers to a composition, which contains the active ingredient and a pharmaceutically acceptable carrier or excipient.

In another embodiment, the HMW-MAA fragment is approximately 98 AA in length. In another embodiment, the length is approximately 194 AA. In another embodiment, the length is approximately 430 AA. In another embodiment, the length is approximately 98-194 AA. In another embodiment, the length is approximately 194-430 AA. In another embodiment, the length is approximately 98-430 AA.

In another embodiment, the length of the HMW-MAA fragment of the present invention is at least 8 amino acids (AA). In another embodiment, the length is more than 8 AA. In another embodiment, the length is at least 9 AA. In another embodiment, the length is more than 9 AA. In another embodiment, the length is at least 10 AA. In another embodiment, the length is more than 10 AA. In another embodiment, the length is at least 11 AA. In another embodiment, the length is more than 11 AA. In another embodiment, the length is at least 12 AA. In another embodiment, the length is more than 12 AA. In another embodiment, the length is at least about 14 AA. In another embodiment, the length is more than 14 AA. In another embodiment, the length is at least about 16 AA. In another embodiment, the length is more than 16 AA. In another embodiment, the length is at least about 18 AA. In another embodiment, the length is more than 18 AA. In another embodiment, the length is at least about 20 AA. In another embodiment, the length is more than 20 AA. In another embodiment, the length is at least about 25 AA. In another embodiment, the length is more than 25 AA. In another embodiment, the length is at least about 30 AA. In another embodiment, the length is more than 30 AA. In another embodiment, the length is at least about 40 AA. In another embodiment, the length is more than 40 AA. In another embodiment, the length is at least about 50 AA. In another embodiment, the length is more than 50 AA. In another embodiment, the length is at least about 70 AA. In another embodiment, the length is more than 70 AA. In another embodiment, the length is at least about 100 AA. In another embodiment, the length is more than 100 AA. In another embodiment, the length is at least about 150 AA. In another embodiment, the length is more than 150 AA. In another embodiment, the length is at least about 200 AA. In another embodiment, the length is more than 200 AA. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the length is about 8-50 AA. In another embodiment, the length is about 8-70 AA. In another embodiment, the length is about 8-100 AA. In another embodiment, the length is about 8-150 AA. In another embodiment, the length is about 8-200 AA. In another embodiment, the length is about 8-250 AA. In another embodiment, the length is about 8-300 AA. In another embodiment, the length is about 8-400 AA. In another embodiment, the length is about 8-500 AA. In another embodiment, the length is about 9-50 AA. In another embodiment, the length is about 9-70 AA. In another embodiment, the length is about 9-100 AA. In another embodiment, the length is about 9-150 AA. In another embodiment, the length is about 9-200 AA. In another embodiment, the length is about 9-250 AA. In another embodiment, the length is about 9-300 AA. In another embodiment, the length is about 10-50 AA. In another embodiment, the length is about 10-70 AA. In another embodiment, the length is about 10-100 AA. In another embodiment, the length is about 10-150 AA. In another embodiment, the length is about 10-200 AA. In another embodiment, the length is about 10-250 AA. In another embodiment, the length is about 10-300 AA. In another embodiment, the length is about 10-400 AA. In another embodiment, the length is about 10-500 AA. In another embodiment, the length is about 11-50 AA. In another embodiment, the length is about 11-70 AA. In another embodiment, the length is about 11-100 AA. In

another embodiment, the length is about 11-150 AA. In another embodiment, the length is about 11-200 AA. In another embodiment, the length is about 11-250 AA. In another embodiment, the length is about 11-300 AA. In another embodiment, the length is about 11-400 AA. In another embodiment, the length is about 11-500 AA. In another embodiment, the length is about 12-50 AA. In another embodiment, the length is about 12-70 AA. In another embodiment, the length is about 12-100 AA. In another embodiment, the length is about 12-150 AA. In another embodiment, the length is about 12-200 AA. In another embodiment, the length is about 12-250 AA. In another embodiment, the length is about 12-300 AA. In another embodiment, the length is about 12-400 AA. In another embodiment, the length is about 12-500 AA. In another embodiment, the length is about 15-50 AA. In another embodiment, the length is about 15-70 AA. In another embodiment, the length is about 15-100 AA. In another embodiment, the length is about 15-150 AA. In another embodiment, the length is about 15-200 AA. In another embodiment, the length is about 15-250 AA. In another embodiment, the length is about 15-300 AA. In another embodiment, the length is about 15-400 AA. In another embodiment, the length is about 15-500 AA. In another embodiment, the length is about 8-400 AA. In another embodiment, the length is about 8-500 AA. In another embodiment, the length is about 20-50 AA. In another embodiment, the length is about 20-70 AA. In another embodiment, the length is about 20-100 AA. In another embodiment, the length is about 20-150 AA. In another embodiment, the length is about 20-200 AA. In another embodiment, the length is about 20-250 AA. In another embodiment, the length is about 20-300 AA. In another embodiment, the length is about 20-400 AA. In another embodiment, the length is about 20-500 AA. In another embodiment, the length is about 30-50 AA. In another embodiment, the length is about 30-70 AA. In another embodiment, the length is about 30-100 AA. In another embodiment, the length is about 30-150 AA. In another embodiment, the length is about 30-200 AA. In another embodiment, the length is about 30-250 AA. In another embodiment, the length is about 30-300 AA. In another embodiment, the length is about 30-400 AA. In another embodiment, the length is about 30-500 AA. In another embodiment, the length is about 40-50 AA. In another embodiment, the length is about 40-70 AA. In another embodiment, the length is about 40-100 AA. In another embodiment, the length is about 40-150 AA. In another embodiment, the length is about 40-200 AA. In another embodiment, the length is about 40-250 AA. In another embodiment, the length is about 40-300 AA. In another embodiment, the length is about 40-400 AA. In another embodiment, the length is about 40-500 AA. In another embodiment, the length is about 50-70 AA. In another embodiment, the length is about 50-100 AA. In another embodiment, the length is about 50-150 AA. In another embodiment, the length is about 50-200 AA. In another embodiment, the length is about 50-250 AA. In another embodiment, the length is about 50-300 AA. In another embodiment, the length is about 50-400 AA. In another embodiment, the length is about 50-500 AA. In another embodiment, the length is about 70-100 AA. In another embodiment, the length is about 70-150 AA. In another embodiment, the length is about 70-200 AA. In another embodiment, the length is about 70-250 AA. In another embodiment, the length is about 70-300 AA. In another embodiment, the length is about 70-400 AA. In another embodiment, the length is about 70-500 AA. In another embodiment, the length is about 100-150 AA. In another embodiment, the length is about 100-200 AA. In another embodiment, the length is about 100-250 AA. In another embodiment, the length is about 100-300 AA. In another embodiment, the length is about 100-400 AA. In another embodiment, the length is about 100-500 AA. Each possibility represents a separate embodiment of the present invention.

Each HMW-MAA protein and each fragment thereof represents a separate embodiment of the present invention.

In another embodiment, a recombinant polypeptide of the methods and compositions of the present invention comprises a signal sequence. In another embodiment, the signal sequence is from the organism used to construct the vaccine vector. In another embodiment, the signal sequence is a LLO signal sequence. In another embodiment, the signal sequence is an ActA signal sequence. In another embodiment, the signal sequence is a Listerial signal sequence. In another embodiment, the signal sequence is any other signal sequence known in the art. Each possibility represents a separate embodiment of the present invention.

The terms "peptide" and "recombinant peptide" refer, in another embodiment, to a peptide or polypeptide of any length. In another embodiment, a peptide or recombinant peptide of the present invention has one of the lengths enumerated above for an HMW-MAA fragment. Each possibility represents a separate embodiment of the present invention. In one embodiment, the term "peptide" refers to native peptides (either degradation products, synthetically synthesized peptides or recombinant peptides) and/or peptidomimetics (typically, synthetically synthesized peptides), such as peptoids and semipeptoids which are peptide analogs, which may have, for example, modifications rendering the peptides more stable while in a body or more capable of penetrating into cells. Such modifications include, but are not limited to N terminus modification, C terminus modification, peptide bond modification, including, but not limited to, CH2—NH, CH2—S, CH2—S═O, O═C—NH, CH2—O, CH2—CH2, S═C—NH, CH═CH or CF═CH, backbone modifications, and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C. A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein. Further details in this respect are provided hereinunder.

Peptide bonds (—CO—NH—) within the peptide may be substituted, for example, by N-methylated bonds (—N(CH3)-CO—), ester bonds (—C(R)H—C—O—O—C(R)—N—), ketomethylen bonds (—CO—CH2—), *-aza bonds (—NH—N(R)—CO—), wherein R is any alkyl, e.g., methyl, carba bonds (—CH2—NH—), hydroxyethylene bonds (—CH(OH)—CH2—), thioamide bonds (—CS—NH—), olefinic double bonds (—CH═CH—), retro amide bonds (—NH—CO—), peptide derivatives (—N(R)—CH2—CO—), wherein R is the "normal" side chain, naturally presented on the carbon atom.

These modifications can occur at any of the bonds along the peptide chain and even at several (2-3) at the same time. Natural aromatic amino acids, Trp, Tyr and Phe, may be substituted for synthetic non-natural acid such as TIC, naphthylelanine (Nol), ring-methylated derivatives of Phe, halogenated derivatives of Phe or o-methyl-Tyr.

In addition to the above, the peptides of the present invention may also include one or more modified amino acids or one or more non-amino acid monomers (e.g. fatty acids, complex carbohydrates etc).

In one embodiment, the term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally in vivo, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" may include both D- and L-amino acids.

Peptides or proteins of this invention may be prepared by various techniques known in the art, including phage display libraries (Hoogenboom and Winter, J. Mol. Biol. 227:381 (1991); Marks et al., J. Mol. Biol. 222:581 (1991)).

In one embodiment, the term "oligonucleotide" is interchangeable with the term "nucleic acid", and may refer to a molecule, which may include, but is not limited to, prokaryotic sequences, eukaryotic mRNA, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. The term also refers to sequences that include any of the known base analogs of DNA and RNA.

In another embodiment, the present invention provides a vaccine comprising a recombinant *Listeria* strain of the present invention. In one embodiment, the vaccine additionally comprises an adjuvant. In one embodiment, the vaccine additionally comprises a cytokine, chemokine, or combination thereof. In one embodiment, a vaccine is a composition which elicits an immune response to an antigen or polypeptide in the composition as a result of exposure to the composition. In another embodiment, the vaccine or composition additionally comprises APCs, which in one embodiment are autologous, while in another embodiment, they are allogeneic to the subject.

In another embodiment, the present invention provides a vaccine comprising a recombinant polypeptide of the present invention and an adjuvant.

In another embodiment, the present invention provides an immunogenic composition comprising a recombinant polypeptide of the present invention. In another embodiment, the immunogenic composition of methods and compositions of the present invention comprises a recombinant vaccine vector encoding a recombinant peptide of the present invention. In another embodiment, the immunogenic composition comprises a plasmid encoding a recombinant peptide of the present invention. In another embodiment, the immunogenic composition comprises an adjuvant. In one embodiment, a vector of the present invention may be administered as part of a vaccine composition. Each possibility represents a separate embodiment of the present invention.

The immunogenic composition utilized in methods and compositions of the present invention comprises, in another embodiment, a recombinant vaccine vector. In another embodiment, the recombinant vaccine vector comprises a recombinant peptide of the present invention. In another embodiment, the recombinant vaccine vector comprises an isolated nucleic acid of the present invention. In another embodiment, the recombinant vaccine vector comprises an isolated nucleic acid encoding a recombinant peptide of the present invention. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a recombinant vaccine vector encoding a recombinant polypeptide of the present invention. In another embodiment, the present invention provides a recombinant vaccine vector comprising a recombinant polypeptide of the present invention. In another embodiment, the expression vector is a plasmid. Methods for constructing and utilizing recombinant vectors are well known in the art and are described, for example, in Sambrook et al. (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Brent et al. (2003, Current Protocols in Molecular Biology, John Wiley & Sons, New York). Each possibility represents a separate embodiment of the present invention.

In another embodiment, the vector is an intracellular pathogen. In another embodiment, the vector is derived from a cytosolic pathogen. In another embodiment, the vector is derived from an intracellular pathogen. In another embodiment, an intracellular pathogen induces a predominantly cell-mediated immune response. In another embodiment, the vector is a *Salmonella* strain. In another embodiment, the vector is a BCG strain. In another embodiment, the vector is a bacterial vector. In another embodiment, dendritic cells transduced with a vector of the present invention may be administered to the subject to upregulate the subject's immune response, which in one embodiment is accomplished by upregulating CTL activity.

In another embodiment, the recombinant vaccine vector induces a predominantly Th1-type immune response.

An immunogenic composition of methods and compositions of the present invention comprises, in another embodiment, an adjuvant that favors a predominantly Th1-type immune response. In another embodiment, the adjuvant favors a predominantly Th1-mediated immune response. In another embodiment, the adjuvant favors a Th1-type immune response. In another embodiment, the adjuvant favors a Th1-mediated immune response. In another embodiment, the adjuvant favors a cell-mediated immune response over an antibody-mediated response. In another embodiment, the adjuvant is any other type of adjuvant known in the art. In another embodiment, the immunogenic composition induces the formation of a T cell immune response against the target protein. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the adjuvant is MPL. In another embodiment, the adjuvant is QS21. In another embodiment, the adjuvant is a TLR agonist. In another embodiment, the adjuvant is a TLR4 agonist. In another embodiment, the adjuvant is a TLR9 agonist. In another embodiment, the adjuvant is Resiquimod®. In another embodiment, the adjuvant is imiquimod. In another embodiment, the adjuvant is a CpG oligonucleotide. In another embodiment, the adjuvant is a cytokine or a nucleic acid encoding same. In another embodiment, the adjuvant is a chemokine or a nucleic acid encoding same. In another embodiment, the adjuvant is IL-12 or a nucleic acid encoding same. In another embodiment, the adjuvant is IL-6 or a nucleic acid encoding same. In another embodiment, the adjuvant is a lipopolysaccharide. In another embodiment, the adjuvant is as described in Fundamental Immunology, 5th ed (August 2003): William E. Paul (Editor); Lippincott Williams & Wilkins Publishers; Chapter 43: Vaccines, G J V Nossal, which is hereby incorporated by reference. In another embodiment, the adjuvant is any other adjuvant known in the art. Each possibility represents a separate embodiment of the present invention. In one embodiment, a "predominantly Th1-type immune response" refers to an immune response in which IFN-gamma is secreted. In another embodiment, it refers to an immune response in which tumor necrosis facto-β is secreted. In another embodiment, it refers to an immune response in which IL-2 is secreted. Each possibility represents a separate embodiment of the present invention. In another embodiment, the vector is selected from *Salmonella* sp., *Shigella* sp., BCG, *L. monocytogenes* (which embodiment is exemplified in Example 2), *E. coli*, and *S. gordonii*. In another embodiment, the fusion pro-

teins are delivered by recombinant bacterial vectors modified to escape phagolysosomal fusion and live in the cytoplasm of the cell. In another embodiment, the vector is a viral vector. In other embodiments, the vector is selected from Vaccinia (which embodiment is exemplified in Example 8), Avipox, Adenovirus, AAV, Vaccinia virus NYVAC, Modified vaccinia strain Ankara (MVA), Semliki Forest virus, Venezuelan equine encephalitis virus, herpes viruses, and retroviruses. In another embodiment, the vector is a naked DNA vector. In another embodiment, the vector is any other vector known in the art. Each possibility represents a separate embodiment of the present invention. In another embodiment, the present invention provides an isolated nucleic acid encoding a recombinant polypeptide of the present invention. In one embodiment, the isolated nucleic acid comprises a sequence sharing at least 85% homology with a nucleic acid encoding a recombinant polypeptide of the present invention. In another embodiment, the isolated nucleic acid comprises a sequence sharing at least 90% homology with a nucleic acid encoding a recombinant polypeptide of the present invention. In another embodiment, the isolated nucleic acid comprises a sequence sharing at least 95% homology with a nucleic acid encoding a recombinant polypeptide of the present invention. In another embodiment, the isolated nucleic acid comprises a sequence sharing at least 97% homology with a nucleic acid encoding a recombinant polypeptide of the present invention. In another embodiment, the isolated nucleic acid comprises a sequence sharing at least 99% homology with a nucleic acid encoding a recombinant polypeptide of the present invention.

In another embodiment, the present invention provides a vaccine comprising a recombinant nucleotide molecule of the present invention and an adjuvant. In another embodiment, the present invention provides a recombinant vaccine vector comprising a recombinant nucleotide molecule of the present invention. In another embodiment, the present invention provides a recombinant vaccine vector encoding a recombinant polypeptide of the present invention. In another embodiment, the present invention provides a recombinant vaccine vector comprising a recombinant polypeptide of the present invention. In another embodiment, the expression vector is a plasmid. Methods for constructing and utilizing recombinant vectors are well known in the art and are described, for example, in Sambrook et al. (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Brent et al. (2003, Current Protocols in Molecular Biology, John Wiley & Sons, New York). Each possibility represents a separate embodiment of the present invention.

Methods for preparing peptide vaccines are well known in the art and are described, for example, in EP1408048, United States Patent Application Number 20070154953, and OGASAWARA et al (Proc. Nati. Acad. Sci. USA Vol. 89, pp. 8995-8999, October 1992). In one embodiment, peptide evolution techniques are used to create an antigen with higher immunogenicity. Techniques for peptide evolution are well known in the art and are described, for example in U.S. Pat. No. 6,773,900.

In one embodiment, a vaccine is a composition which elicits an immune response to an antigen or polypeptide in the composition as a result of exposure to the composition.

In another embodiment, the present invention provides a recombinant *Listeria* strain comprising a recombinant nucleotide molecule of the present invention.

The recombinant *Listeria* strain of methods and compositions of the present invention is, in another embodiment, a recombinant *Listeria monocytogenes* strain. In another embodiment, the *Listeria* strain is a recombinant *Listeria seeligeri* strain. In another embodiment, the *Listeria* strain is a recombinant *Listeria grayi* strain. In another embodiment, the *Listeria* strain is a recombinant *Listeria ivanovii* strain. In another embodiment, the *Listeria* strain is a recombinant *Listeria murrayi* strain. In another embodiment, the *Listeria* strain is a recombinant *Listeria welshimeri* strain. In another embodiment, the *Listeria* strain is a recombinant strain of any other *Listeria* species known in the art. In one embodiment, the *Listeria* strain is a *Listeria* strain comprising LLO, while in another embodiment, the *Listeria* strain is a *Listeria* strain comprising ActA, while in another embodiment, the *Listeria* strain is a *Listeria* strain comprising PEST-like sequences.

In another embodiment the *Listeria* strain is attenuated by deletion of a gene. In another embodiment the *Listeria* strain is attenuated by deletion of more than 1 gene. In another embodiment the *Listeria* strain is attenuated by deletion or inactivation of a gene. In another embodiment the *Listeria* strain is attenuated by deletion or inactivation of more than 1 gene.

In another embodiment, the gene that is mutated is hly. In another embodiment, the gene that is mutated is actA. In another embodiment, the gene that is mutated is plc A. In another embodiment, the gene that is mutated is plcB. In another embodiment, the gene that is mutated is mpl. In another embodiment, the gene that is mutated is inl A. In another embodiment, the gene that is mutated is inlB. In another embodiment, the gene that is mutated is bsh.

In another embodiment, the *Listeria* strain is an auxotrophic mutant. In another embodiment, the *Listeria* strain is deficient in a gene encoding a vitamin synthesis gene. In another embodiment, the *Listeria* strain is deficient in a gene encoding pantothenic acid synthase.

In another embodiment, the *Listeria* strain is deficient in an AA metabolism enzyme. In another embodiment the *Listeria* strain is deficient in a D-glutamic acid synthase gene. In another embodiment the *Listeria* strain is deficient in the dat gene. In another embodiment the *Listeria* strain is deficient in the dal gene. In another embodiment the *Listeria* strain is deficient in the dga gene. In another embodiment the *Listeria* strain is deficient in a gene involved in the synthesis of diaminopimelic acid. CysK. In another embodiment, the gene is vitamin-B12 independent methionine synthase. In another embodiment, the gene is trpA. In another embodiment, the gene is trpB. In another embodiment, the gene is trpE. In another embodiment, the gene is asnB. In another embodiment, the gene is gltD. In another embodiment, the gene is gltB. In another embodiment, the gene is leuA. In another embodiment, the gene is argG. In another embodiment, the gene is thrC. In another embodiment, the *Listeria* strain is deficient in one or more of the genes described hereinabove.

In another embodiment, the *Listeria* strain is deficient in a synthase gene. In another embodiment, the gene is an AA synthesis gene. In another embodiment, the gene is folP. In another embodiment, the gene is dihydrouridine synthase family protein. In another embodiment, the gene is ispD. In another embodiment, the gene is ispF. In another embodiment, the gene is phosphoenolpyruvate synthase. In another embodiment, the gene is hisF. In another embodiment, the gene is hisH. In another embodiment, the gene is fliI. In another embodiment, the gene is ribosomal large subunit pseudouridine synthase. In another embodiment, the gene is ispD. In another embodiment, the gene is bifunctional GMP synthase/glutamine amidotransferase protein. In another embodiment, the gene is cobS. In another embodiment, the gene is cobB. In another embodiment, the gene is cbiD. In another embodiment, the gene is uroporphyrin-III C-methyltransferase/uroporphyrinogen-III synthase. In another embodiment, the gene is cobQ. In another embodiment, the

gene is uppS. In another embodiment, the gene is truB. In another embodiment, the gene is dxs. In another embodiment, the gene is mvaS. In another embodiment, the gene is dapA. In another embodiment, the gene is ispG. In another embodiment, the gene is folC. In another embodiment, the gene is citrate synthase. In another embodiment, the gene is argJ. In another embodiment, the gene is 3-deoxy-7-phosphoheptulonate synthase. In another embodiment, the gene is indole-3-glycerol-phosphate synthase. In another embodiment, the gene is anthranilate synthase/glutamine amidotransferase component. In another embodiment, the gene is menB. In another embodiment, the gene is menaquinone-specific isochorismate synthase. In another embodiment, the gene is phosphoribosylformylglycinamidine synthase I or II. In another embodiment, the gene is phosphoribosylaminoimidazole-succinocarboxamide synthase. In another embodiment, the gene is carB. In another embodiment, the gene is carA. In another embodiment, the gene is thyA. In another embodiment, the gene is mgsA. In another embodiment, the gene is aroB. In another embodiment, the gene is hepB. In another embodiment, the gene is rluB. In another embodiment, the gene is ilvB. In another embodiment, the gene is ilvN. In another embodiment, the gene is alsS. In another embodiment, the gene is fabF. In another embodiment, the gene is fabH. In another embodiment, the gene is pseudouridine synthase. In another embodiment, the gene is pyrG. In another embodiment, the gene is truA. In another embodiment, the gene is pabB. In another embodiment, the gene is an atp synthase gene (e.g. atpC, atpD-2, aptG, atpA-2, etc).

In another embodiment, the gene is phoP. In another embodiment, the gene is aroA aroC. In another embodiment, the gene is aroD. In another embodiment, the gene is plcB.

In another embodiment, the *Listeria* strain is deficient in a peptide transporter. In another embodiment, the gene is ABC transporter/ATP-binding/permease protein. In another embodiment, the gene is oligopeptide ABC transporter/oligopeptide-binding protein. In another embodiment, the gene is oligopeptide ABC transporter/permease protein. In another embodiment, the gene is zinc ABC transporter/zinc-binding protein. In another embodiment, the gene is sugar ABC transporter. In another embodiment, the gene is phosphate transporter. In another embodiment, the gene is ZIP zinc transporter. In another embodiment, the gene is drug resistance transporter of the EmrB/QacA family. In another embodiment, the gene is sulfate transporter. In another embodiment, the gene is proton-dependent oligopeptide transporter. In another embodiment, the gene is magnesium transporter. In another embodiment, the gene is formate/nitrite transporter. In another embodiment, the gene is spermidine/putrescine ABC transporter. In another embodiment, the gene is Na/Pi-cotransporter. In another embodiment, the gene is sugar phosphate transporter. In another embodiment, the gene is glutamine ABC transporter. In another embodiment, the gene is major facilitator family transporter. In another embodiment, the gene is glycine betaine/L-proline ABC transporter. In another embodiment, the gene is molybdenum ABC transporter. In another embodiment, the gene is techoic acid ABC transporter. In another embodiment, the gene is cobalt ABC transporter. In another embodiment, the gene is ammonium transporter. In another embodiment, the gene is amino acid ABC transporter. In another embodiment, the gene is cell division ABC transporter. In another embodiment, the gene is manganese ABC transporter. In another embodiment, the gene is iron compound ABC transporter. In another embodiment, the gene is maltose/maltodextrin ABC transporter. In another embodiment, the gene is drug resistance transporter of the Bcr/CflA family.

In another embodiment, the gene is a subunit of one of the above proteins.

In another embodiment, a recombinant *Listeria* strain of the present invention has been passaged through an animal host. In another embodiment, the passaging maximizes efficacy of the strain as a vaccine vector. In another embodiment, the passaging stabilizes the immunogenicity of the *Listeria* strain. In another embodiment, the passaging stabilizes the virulence of the *Listeria* strain. In another embodiment, the passaging increases the immunogenicity of the *Listeria* strain. In another embodiment, the passaging increases the virulence of the *Listeria* strain. In another embodiment, the passaging removes unstable sub-strains of the *Listeria* strain. In another embodiment, the passaging reduces the prevalence of unstable sub-strains of the *Listeria* strain. In another embodiment, the passaging attenuates the strain, or in another embodiment, makes the strain less virulent. Methods for passaging a recombinant *Listeria* strain through an animal host are well known in the art, and are described, for example, in U.S. patent application Ser. No. 10/541,614. Each possibility represents a separate embodiment of the present invention.

Each *Listeria* strain and type thereof represents a separate embodiment of the present invention.

In another embodiment, the recombinant Listeria of methods and compositions of the present invention is stably transformed with a construct encoding an antigen or an LLO-antigen fusion. In one embodiment, the construct contains a polylinker to facilitate further subcloning. Several techniques for producing recombinant *Listeria* are known; each technique represents a separate embodiment of the present invention.

In another embodiment, the construct or heterologous gene is integrated into the Listerial chromosome using homologous recombination. Techniques for homologous recombination are well known in the art, and are described, for example, in Frankel, F R, Hegde, S, Lieberman, J, and Y Paterson. Induction of a cell-mediated immune response to HIV gag using *Listeria monocytogenes* as a live vaccine vector. J. Immunol. 155: 4766-4774. 1995; Mata, M, Yao, Z, Zubair, A, Syres, K and Y Paterson, Evaluation of a recombinant *Listeria monocytogenes* expressing an HIV protein that protects mice against viral challenge. Vaccine 19:1435-45, 2001; Boyer, J D, Robinson, T M, Maciag, P C, Peng, X, Johnson, R S, Paviakis, G, Lewis, M G, Shen, A, Siliciano, R, Brown, C R, Weiner, D, and Y Paterson. DNA prime *Listeria* boost induces a cellular immune response to SIV antigens in the Rhesus Macaque model that is capable of limited suppression of SIV239 viral replication. Virology. 333: 88-101, 2005. In another embodiment, homologous recombination is performed as described in U.S. Pat. No. 6,855,320. In another embodiment, a temperature sensitive plasmid is used to select the recombinants. Each technique represents a separate embodiment of the present invention.

In another embodiment, the construct or heterologous gene is integrated into the Listerial chromosome using transposon insertion. Techniques for transposon insertion are well known in the art, and are described, inter alia, by Sun et al. (Infection and Immunity 1990, 58: 3770-3778) in the construction of DP-L967. Transposon mutagenesis has the advantage, in another embodiment, that a stable genomic insertion mutant can be formed. In another embodiment, the position in the genome where the foreign gene has been inserted by transposon mutagenesis is unknown.

In another embodiment, the construct or heterologous gene is integrated into the Listerial chromosome using phage integration sites (Lauer P, Chow M Y et al, Construction, characterization, and use of two LM site-specific phage integration

vectors. J Bacteriol 2002; 184(15): 4177-86). In another embodiment, an integrase gene and attachment site of a bacteriophage (e.g. U153 or PSA listeriophage) is used to insert the heterologous gene into the corresponding attachment site, which can be any appropriate site in the genome (e.g. comK or the 3' end of the arg tRNA gene). In another embodiment, endogenous prophages are cured from the attachment site utilized prior to integration of the construct or heterologous gene. In another embodiment, this method results in single-copy integrants. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the construct is carried by the *Listeria* strain on a plasmid. LM vectors that express antigen fusion proteins have been constructed via this technique. Lm-GG/E7 was made by complementing a prfA-deletion mutant with a plasmid containing a copy of the prfA gene and a copy of the E7 gene fused to a form of the LLO (hly) gene truncated to eliminate the hemolytic activity of the enzyme, as described herein. Functional LLO was maintained by the organism via the endogenous chromosomal copy of hly. In another embodiment, the plasmid contains an antibiotic resistance gene. In another embodiment, the plasmid contains a gene encoding a virulence factor that is lacking in the genome of the transformed *Listeria* strain. In another embodiment, the virulence factor is prfA. In another embodiment, the virulence factor is LLO. In another embodiment, the virulence factor is ActA. In another embodiment, the virulence factor is any of the genes enumerated above as targets for attenuation. In another embodiment, the virulence factor is any other virulence factor known in the art. Each possibility represents a separate embodiment of the present invention.

In another embodiment, a recombinant peptide of the present invention is fused to a Listerial protein, such as PI-PLC, or a construct encoding same. In another embodiment, a signal sequence of a secreted Listerial protein such as hemolysin, ActA, or phospholipases is fused to the antigen-encoding gene. In another embodiment, a signal sequence of the recombinant vaccine vector is used. In another embodiment, a signal sequence functional in the recombinant vaccine vector is used. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the construct is contained in the *Listeria* strain in an episomal fashion. In another embodiment, the foreign antigen is expressed from a vector harbored by the recombinant *Listeria* strain.

Each method of expression in *Listeria* represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of inducing an anti-HMW-MAA immune response in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby inducing an anti-HMW-MAA immune response in a subject.

In another embodiment, a subject is administered his/her own allogeneic cells, which in one embodiment, elicit an immune response to an antigen. In another embodiment, the compositions and methods of the present invention result in the expression of stimulatory cytokines, which in one embodiment, are Th1 cytokines, which in one embodiment, is IFN-gamma. In one embodiment, the expression of stimulatory cytokines contributes to the anti-tumor effect of the compositions and methods. In another embodiment, the compositions and methods of the present invention result in the expression of gamma delta T cells.

In another embodiment, the present invention provides compositions and methods for inducing non-specific anti-tumor responses. In one embodiment, immunization with a melanoma antigen, such as HMW-MAA peptide, protects against a type of melanoma that does not express the antigen (Example 4).

In another embodiment, the present invention provides a method of inducing an immune response against an HMW-MAA-expressing tumor in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby inducing an immune response against an HMW-MAA-expressing tumor. As provided herein, vaccines of the present invention induce antigen-specific immune response, as shown by multiple lines of evidence—e.g. inhibition of tumor growth, tetramer staining, measurement of numbers of tumor-infiltrating $CD8^+$ T cells, FACS, and chromium release assay.

In another embodiment, the present invention provides a method of inducing an immune response against a pericyte in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby inducing an immune response against a pericyte.

In another embodiment, the present invention provides a method of impeding the growth of a solid tumor in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby impeding a growth and/or delaying progression of a solid tumor in a subject. In another embodiment, the subject mounts an immune response against a pericyte of the solid tumor. In another embodiment, the pericyte is in a vasculature of the solid tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of treating a solid tumor in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby treating a solid tumor in a subject. In another embodiment, the subject mounts an immune response against a pericyte of the solid tumor. In another embodiment, the pericyte is in a vasculature of the solid tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of lysing one or more tumor cells in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby lysing one or more tumor cells in a subject. In one embodiment, tumor lysis is due to cytotoxic T lymphocytes, tumor infilitrating lymphocytes, or a combination thereof, which in one embodiment are tumor-specific.

In one embodiment, methods of the present invention are used to treat, impede, suppress, inhibit, or prevent any of the above-described diseases, disorders, symptoms, or side effects associated with allergy or asthma. In one embodiment, "treating" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or lessen the targeted pathologic condition or disorder as described hereinabove. Thus, in one embodiment, treating may include directly affecting or curing, suppressing, inhibiting, preventing, reducing the severity of, delaying the onset of, reducing symptoms associated with the disease, disorder or condition, or a combination thereof. Thus, in one embodiment, "treating" refers inter alia to delaying progression, expediting remission, inducing remission, augmenting remission, speeding recovery, increasing efficacy of or decreasing resistance to alternative therapeutics, or a combination thereof. In one embodiment, "preventing" or "impeding" refers, inter alia, to delaying the onset of symptoms, preventing relapse to a disease, decreasing the number or frequency of relapse episodes, increasing latency between

symptomatic episodes, or a combination thereof. In one embodiment, "suppressing" or "inhibiting", refers inter alia to reducing the severity of symptoms, reducing the severity of an acute episode, reducing the number of symptoms, reducing the incidence of disease-related symptoms, reducing the latency of symptoms, ameliorating symptoms, reducing secondary symptoms, reducing secondary infections, prolonging patient survival, or a combination thereof.

In one embodiment, symptoms are primary, while in another embodiment, symptoms are secondary. In one embodiment, "primary" refers to a symptom that is a direct result of a particular disease or disorder, while in one embodiment, "secondary" refers to a symptom that is derived from or consequent to a primary cause. In one embodiment, the compounds for use in the present invention treat primary or secondary symptoms or secondary complications related to allergy or asthma. In another embodiment, "symptoms" may be any manifestation of a disease or pathological condition.

As provided herein, *Listeria* strains expressing HMW-MAA inhibited growth of tumors that did not express HMW-MAA. These findings show that anti-HMW-MAA immune responses inhibit and reverse vascularization of, and thus inhibit growth of, solid tumors. Anti-HMW-MAA vaccines of the present invention were able to exert these effects in spite of the incomplete identity (80%) between HMW-MAA and its mouse homolog, nameely mouse chondroitin sulfate proteoglycan ("AN2"). In this embodiment, anti-HMW-MAA vaccines of the present invention are efficacious for vaccination against any solid tumor, regardless of its expression of HMW-MAA. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of impeding a vascularization of a solid tumor in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby impeding a vascularization of a solid tumor in a subject. In another embodiment, the subject mounts an immune response against a pericyte of the solid tumor. In another embodiment, the pericyte is in a vasculature of the solid tumor. Each possibility represents a separate embodiment of the present invention.

The solid tumor that is the target of methods and compositions of the present invention is, in another embodiment, a melanoma. In another embodiment, the tumor is a sarcoma. In another embodiment, the tumor is a carcinoma. In another embodiment, the tumor is a mesothelioma (e.g. malignant mesothelioma). In another embodiment, the tumor is a glioma. In another embodiment, the tumor is a germ cell tumor. In another embodiment, the tumor is a choriocarcinoma.

In another embodiment, the tumor is pancreatic cancer. In another embodiment, the tumor is ovarian cancer. In another embodiment, the tumor is gastric cancer. In another embodiment, the tumor is a carcinomatous lesion of the pancreas. In another embodiment, the tumor is pulmonary adenocarcinoma. In another embodiment, the tumor is colorectal adenocarcinoma. In another embodiment, the tumor is pulmonary squamous adenocarcinoma. In another embodiment, the tumor is gastric adenocarcinoma. In another embodiment, the tumor is an ovarian surface epithelial neoplasm (e.g. a benign, proliferative or malignant variety thereof). In another embodiment, the tumor is an oral squamous cell carcinoma. In another embodiment, the tumor is non small-cell lung carcinoma. In another embodiment, the tumor is an endometrial carcinoma. In another embodiment, the tumor is a bladder cancer. In another embodiment, the tumor is a head and neck cancer. In another embodiment, the tumor is a prostate carcinoma.

In another embodiment, the tumor is a non-small cell lung cancer (NSCLC). In another embodiment, the tumor is a Wilms' tumor. In another embodiment, the tumor is a desmoplastic small round cell tumor. In another embodiment, the tumor is a colon cancer. In another embodiment, the tumor is a lung cancer. In another embodiment, the tumor is an ovarian cancer. In another embodiment, the tumor is a uterine cancer. In another embodiment, the tumor is a thyroid cancer. In another embodiment, the tumor is a hepatocellular carcinoma. In another embodiment, the tumor is a thyroid cancer. In another embodiment, the tumor is a liver cancer. In another embodiment, the tumor is a renal cancer. In another embodiment, the tumor is a kaposis. In another embodiment, the tumor is a sarcoma. In another embodiment, the tumor is another carcinoma or sarcoma. Each possibility represents a separate embodiment of the present invention.

In one embodiment, this invention provides compositions and methods for preventing cancer in populations that are predisposed to the cancer or in populations that are at high risk for the cancer, which in one embodiment, may be a population of women with brcal or 2 mutations, which population in one embodiment is susceptible to breast cancer.

Each of the above types of cancer represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of inducing an immune response against an HMW-MAA-expressing tumor in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby inducing an immune response against an HMW-MAA-expressing tumor. As provided herein, *Listeria* strains expressing HMW-MAA elicited anti-HMW-MAA immune responses and inhibited growth HMW-MAA-expressing of tumors.

The HMW-MAA-expressing tumor that is the target of methods and compositions of the present invention is, in another embodiment, a basal cell carcinoma. In another embodiment, the HMW-MAA-expressing tumor is a tumor of neural crest origin. In another embodiment, the HMW-MAA-expressing tumor is an astrocytoma. In another embodiment, the HMW-MAA-expressing tumor is a glioma. In another embodiment, the HMW-MAA-expressing tumor is a neuroblastoma. In another embodiment, the HMW-MAA-expressing tumor is a sarcoma. In another embodiment, the HMW-MAA-expressing tumor is a childhood leukemia. In another embodiment, the HMW-MAA-expressing tumor is a lobular breast carcinoma lesion. In another embodiment, the HMW-MAA-expressing tumor is a melanoma. In another embodiment, the HMW-MAA-expressing tumor is any other HMW-MAA-expressing tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method ofinducingan immuneresponse against a pericyte in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby inducing an immune response against a pericyte. As provided herein, *Listeria* strains expressing HMW-MAA inhibited growth of solid tumors, even those that did not express HMW-MAA. These findings demonstrate inhibition of vascularization via induction of immune responses against tumor-vascular associated pericytes.

In another embodiment, the present invention provides a method of impeding a growth and/or delaying progression of a HMW-MAA-expressing tumor in a subject, comprising administering to the subject a composition comprising a

recombinant *Listeria* strain of the present invention, thereby impeding a growth and/or delaying progression of a HMW-MAA-expressing tumor in a subject. In another embodiment, the subject mounts an immune response against the HMW-MAA-expressing tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of treating a HMW-MAA-expressing tumor in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* strain of the present invention, thereby treating a HMW-MAA-expressing tumor in a subject. In another embodiment, the subject mounts an immune response against the HMW-MAA-expressing tumor. Each possibility represents a separate embodiment of the present invention.

The recombinant *Listeria* strain of the present invention utilized in methods of the present invention comprises, in another embodiment, a recombinant polypeptide of the present invention. In another embodiment, the recombinant *Listeria* strain comprises a recombinant nucleotide molecule of the present invention. In another embodiment, the recombinant *Listeria* strain is any recombinant *Listeria* strain described or enumerated above. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of inducing an anti-HMW-MAA immune response in a subject, comprising administering to the subject a composition comprising a recombinant polypeptide of the present invention, thereby inducing an anti-HMW-MAA immune response in a subject.

In another embodiment, the present invention provides a method of inducing an immune response against an HMW-MAA-expressing tumor in a subject, comprising administering to the subject a composition comprising a recombinant polypeptide of the present invention, thereby inducing an immune response against an HMW-MAA-expressing tumor.

In another embodiment, the present invention provides a method of inducing an immune response against a pericyte in a subject, comprising administering to the subject a composition comprising a recombinant polypeptide of the present invention, thereby inducing an immune response against a pericyte.

In another embodiment, the present invention provides a method of impeding a growth and/or delaying progression of a solid tumor in a subject, comprising administering to the subject a composition comprising a recombinant polypeptide of the present invention, thereby impeding a growth and/or delaying progression of a solid tumor in a subject. In another embodiment, the subject mounts an immune response against a pericyte of the solid tumor. In another embodiment, the pericyte is in a vasculature of the solid tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of treating a solid tumor in a subject, comprising administering to the subject a composition comprising a recombinant polypeptide of the present invention, thereby treating a solid tumor in a subject. In another embodiment, the subject mounts an immune response against a pericyte of the solid tumor. In another embodiment, the pericyte is in a vasculature of the solid tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of impeding a vascularization of a solid tumor in a subject, comprising administering to the subject a composition comprising a recombinant polypeptide of the present invention, thereby impeding a vascularization of a solid tumor in a subject. In another embodiment, the subject mounts an immune response against a pericyte of the solid tumor. In another embodiment, the pericyte is in a vasculature of the solid tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of impeding a growth and/or delaying progression of a HMW-MAA-expressing tumor in a subject, comprising administering to the subject a composition comprising a recombinant polypeptide of the present invention, thereby impeding a growth and/or delaying progression of a HMW-MAA-expressing tumor in a subject. In another embodiment, the subject mounts an immune response against the HMW-MAA-expressing tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of treating a HMW-MAA-expressing tumor in a subject, comprising administering to the subject a composition comprising a recombinant polypeptide of the present invention, thereby treating a HMW-MAA-expressing tumor in a subject. In another embodiment, the subject mounts an immune response against the HMW-MAA-expressing tumor. Each possibility represents a separate embodiment of the present invention.

In other embodiments, the recombinant polypeptide of any of the methods described above have any of the characteristics of a recombinant polypeptide of compositions of the present invention. Each characteristic represents a separate embodiment of the present invention.

In another embodiment of methods of the present invention, a vaccine comprising a recombinant *Listeria* strain of the present invention is administered. In another embodiment, an immunogenic composition comprising a recombinant *Listeria* strain of the present invention is administered. In another embodiment, a vaccine comprising a recombinant polypeptide of the present invention is administered. In another embodiment, an immunogenic composition comprising a recombinant polypeptide of the present invention is administered. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the target pericyte of methods and compositions of the present invention is an activated pericyte. In another embodiment, the target pericyte is any other type of pericyte known in the art. Each possibility represents a separate embodiment of the present invention.

In another embodiment, a method or immunogenic composition of methods and compositions of the present invention induces a cell-mediated immune response. In another embodiment, the immunogenic composition induces a predominantly cell-mediated immune response. In another embodiment, the immunogenic composition induces a predominantly Th1-type immune response. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the immune response elicited by methods of the present invention is a cell-mediated immune response. In another embodiment, the immune response is a T-cell-mediated immune response. Each possibility represents a separate embodiment of the present invention.

The T cell-mediated immune response induced by methods and compositions of the present invention comprises, in another embodiment, a CTL. In another embodiment, the T cell involved in the T cell-mediated immune response is a CTL. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the T cell-mediated immune response comprises a T helper cell. In another embodiment,

the T cell involved in the T cell-mediated immune response is a T helper cell. Each possibility represents a separate embodiment of the present invention.

In another embodiment of methods of the present invention, the subject is immunized with an immunogenic composition, vector, or recombinant peptide of the present invention. In another embodiment, the subject is contacted with the immunogenic composition, vector, or recombinant peptide. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of inhibiting adhesion of a cancer cell to the extracellular matrix, comprising inducing an anti-HMW-MAA immune response by a method of the present invention, thereby inhibiting adhesion of a cancer cell to the extracellular matrix. In another embodiment, the cancer cell is a melanoma cell. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of inhibiting metastasis of a tumor, comprising inducing an anti-HMW-MAA immune response by a method of the present invention, thereby inhibiting metastasis of a tumor. In another embodiment, the tumor is a melanoma tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of inhibiting migration of a cancer cell, comprising inducing an anti-HMW-MAA immune response by a method of the present invention, thereby inhibiting migration of a cancer cell. In another embodiment, the cancer cell is a melanoma cell. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of inhibiting proliferation of cells in a tumor, comprising inducing an anti-HMW-MAA immune response by a method of the present invention, thereby inhibiting proliferation of cells in a tumor. In another embodiment, the tumor is a melanoma tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of reducing invasiveness of a tumor, comprising inducing an anti-HMW-MAA immune response by a method of the present invention, thereby reducing invasiveness of a tumor. In another embodiment, the tumor is a melanoma tumor. In another embodiment, anti-HMW-MAA immune responses inhibit formation of HMW-MAA-MT3-MMP (membrane type metalloproteinases) complexes. In another embodiment, inhibition of formation of these complexes inhibits degradation of type I collagen by melanoma cells. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of inhibiting conversion of plasminogen into plasmin in the vicinity of a tumor, comprising inducing an anti-HMW-MAA immune response by a method of the present invention, thereby inhibiting conversion of plasminogen into plasmin in the vicinity of a tumor. In another embodiment, the tumor is a melanoma tumor. In another embodiment, inhibiting plasmin release inhibits, in turn, degradation of the extracellular matrix (ECM). Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a method of inhibiting sequestration of angiostatin in the vicinity of a tumor, comprising inducing an anti-HMW-MAA immune response by a method of the present invention, thereby inhibiting sequestration of angiostatin in the vicinity of a tumor. In another embodiment, the tumor is a melanoma tumor. Each possibility represents a separate embodiment of the present invention.

In another embodiment, a peptide of the present invention is homologous to a peptide enumerated herein. The terms "homology," "homologous," etc, when in reference to any protein or peptide, refer, in one embodiment, to a percentage of amino acid residues in the candidate sequence that are identical with the residues of a corresponding native polypeptide, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. Methods and computer programs for the alignment are well known in the art.

Homology is, in another embodiment, determined by computer algorithm for sequence alignment, by methods well described in the art. For example, computer algorithm analysis of nucleic acid sequence homology can include the utilization of any number of software packages available, such as, for example, the BLAST, DOMAIN, BEAUTY (BLAST Enhanced Alignment Utility), GENPEPT and TREMBL packages.

In another embodiment, "homology" or "homologous" refers to identity to a non-HMW-MAA sequence selected from SEQ ID No: 1-14 of greater than 70%. In another embodiment, "homology" refers to identity to a sequence selected from SEQ ID No: 1-14 of greater than 72%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 1-14 of greater than 75%. In another embodiment, "homology" refers to identity to a sequence selected from SEQ ID No: 1-14 of greater than 78%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 1-14 of greater than 80%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 1-14 of greater than 82%. In another embodiment, "homology" refers to identity to a sequence selected from SEQ ID No: 1-14 of greater than 83%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 1-14 of greater than 85%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 1-14 of greater than 87%. In another embodiment, "homology" refers to identity to a sequence selected from SEQ ID No: 1-14 of greater than 88%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 1-14 of greater than 90%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 1-14 of greater than 92%. In another embodiment, "homology" refers to identity to a sequence selected from SEQ ID No: 1-14 of greater than 93%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 1-14 of greater than 95%. In another embodiment, "homology" refers to identity to a sequence selected from SEQ ID No: 1-14 of greater than 96%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 1-14 of greater than 97%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 1-14 of greater than 98%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 1-14 of greater than 99%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 1-14 of 100%. Each possibility represents a separate embodiment of the present invention.

In another embodiment, "homology" or "homologous" refers to identity to an HMW-MAA sequence selected from SEQ ID No: 15-16 of greater than 70%. In another embodiment, "homology" refers to identity to a sequence selected from SEQ ID No: 15-16 of greater than 72%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 15-16 of greater than 75%. In another embodiment,

"homology" refers to identity to a sequence selected from SEQ ID No: 15-16 of greater than 78%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 15-16 of greater than 80%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 15-16 of greater than 82%. In another embodiment, "homology" refers to identity to a sequence selected from SEQ ID No: 15-16 of greater than 83%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 15-16 of greater than 85%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 15-16 of greater than 87%. In another embodiment, "homology" refers to identity to a sequence selected from SEQ ID No: 15-16 of greater than 88%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 15-16 of greater than 90%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 15-16 of greater than 92%. In another embodiment, "homology" refers to identity to a sequence selected from SEQ ID No: 15-16 of greater than 93%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 15-16 of greater than 95%. In another embodiment, "homology" refers to identity to a sequence selected from SEQ ID No: 15-16 of greater than 96%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 15-16 of greater than 97%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 15-16 of greater than 98%. In another embodiment, "homology" refers to identity to one of SEQ ID No: 15-16 of greater than 99%. In another embodiment; "homology" refers to identity to one of SEQ ID No: 15-16 of 100%. Each possibility represents a separate embodiment of the present invention.

In another embodiment, homology is determined via determination of candidate sequence hybridization, methods of which are well described in the art (See, for example, "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., Eds. (1985); Sambrook et al., 2001, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y). In other embodiments, methods of hybridization are carried out under moderate to stringent conditions, to the complement of a DNA encoding a native caspase peptide. Hybridization conditions being, for example, overnight incubation at 42° C. in a solution comprising: 10-20% formamide, 5×SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5×Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA.

Protein and/or peptide homology for any AA sequence listed herein is determined, in another embodiment, by methods well described in the art, including immunoblot analysis, or via computer algorithm analysis of AA sequences, utilizing any of a number of software packages available, via established methods. Some of these packages include the FASTA, BLAST, MPsrch or Scanps packages, and, in another embodiment, employ the use of the Smith and Waterman algorithms, and/or globalaocal or BLOCKS alignments for analysis. Each method of determining homology represents a separate embodiment of the present invention.

In another embodiment of the present invention, "nucleic acids" or "nucleotide" refers to a string of at least two base-sugar-phosphate combinations. The term includes, in one embodiment, DNA and RNA. "Nucleotides" refers, in one embodiment, to the monomeric units of nucleic acid polymers. RNA is, in one embodiment, in the form of a tRNA (transfer RNA), snRNA (small nuclear RNA), rRNA (ribosomal RNA), mRNA (messenger RNA), anti-sense RNA, small inhibitory RNA (siRNA), micro RNA (miRNA) and ribozymes. The use of siRNA and miRNA has been described (Caudy A A et al, Genes & Devel 16: 2491-96 and references cited therein). DNA can be, in other embodiments, in form of plasmid DNA, viral DNA, linear DNA, or chromosomal DNA or derivatives of these groups. In addition, these forms of DNA and RNA can be single, double, triple, or quadruple stranded. The term also includes, in another embodiment, artificial nucleic acids that contain other types of backbones but the same bases. In one embodiment, the artificial nucleic acid is a PNA (peptide nucleic acid). PNA contain peptide backbones and nucleotide bases and are able to bind, in one embodiment, to both DNA and RNA molecules. In another embodiment, the nucleotide is oxetane modified. In another embodiment, the nucleotide is modified by replacement of one or more phosphodiester bonds with a phosphorothioate bond. In another embodiment, the artificial nucleic acid contains any other variant of the phosphate backbone of native nucleic acids known in the art. The use of phosphothiorate nucleic acids and PNA are known to those skilled in the art, and are described in, for example, Neilsen P E, Curr Opin Struct Biol 9:353-57; and Raz N K et al Biochem Biophys Res Commun. 297:1075-84. The production and use of nucleic acids is known to those skilled in art and is described, for example, in Molecular Cloning, (2001), Sambrook and Russell, eds. and Methods in Enzymology: Methods for molecular cloning in eukaryotic cells (2003) Purchio and G. C. Fareed. Each nucleic acid derivative represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a kit comprising a compound or composition utilized in performing a method of the present invention. In another embodiment, the present invention provides a kit comprising a composition, tool, or instrument of the present invention. Each possibility represents a separate embodiment of the present invention.

Pharmaceutical Compositions and Methods of Administration

"Pharmaceutical composition" refers, in another embodiment, to a therapeutically effective amount of the active ingredient, i.e. the recombinant peptide or vector comprising or encoding same, together with a pharmaceutically acceptable carrier or diluent. A "therapeutically effective amount" refers, in another embodiment, to that amount which provides a therapeutic effect for a given condition and administration regimen.

The pharmaceutical compositions containing the active ingredient can be, in another embodiment, administered to a subject by any method known to a person skilled in the art, such as parenterally, transmucosally, transdermally, intramuscularly, intravenously, intra-dermally, subcutaneously, intra-peritonealy, intra-ventricularly, intra-cranially, intravaginally, or intra-tumorally.

In another embodiment of methods and compositions of the present invention, the pharmaceutical compositions are administered orally, and are thus formulated in a form suitable for oral administration, i.e. as a solid or a liquid preparation. Suitable solid oral formulations include tablets, capsules, pills, granules, pellets and the like. Suitable liquid oral formulations include solutions, suspensions, dispersions, emulsions, oils and the like. In another embodiment of the present invention, the active ingredient is formulated in a capsule. In accordance with this embodiment, the compositions of the present invention comprise, in addition to the active compound and the inert carrier or diluent, a hard gelating capsule.

In another embodiment, the pharmaceutical compositions are administered by intravenous, intra-arterial, or intra-muscular injection of a liquid preparation. Suitable liquid formulations include solutions, suspensions, dispersions, emulsions, oils and the like. In another embodiment, the pharmaceutical compositions are administered intravenously and are thus formulated in a form suitable for intravenous administration. In another embodiment, the pharmaceutical compositions are administered intra-arterially and are thus formulated in a form suitable for intra-arterial administration. In another embodiment, the pharmaceutical compositions are administered intra-muscularly and are thus formulated in a form suitable for intra-muscular administration.

In another embodiment, the pharmaceutical compositions are administered topically to body surfaces and are thus formulated in a form suitable for topical administration. Suitable topical formulations include gels, ointments, creams, lotions, drops and the like. For topical administration, the recombinant peptide or vector is prepared and applied as a solution, suspension, or emulsion in a physiologically acceptable diluent with or without a pharmaceutical carrier.

In another embodiment, the active ingredient is delivered in a vesicle, e.g. a liposome.

In other embodiments, carriers or diluents used in methods of the present invention include, but are not limited to, a gum, a starch (e.g. corn starch, pregeletanized starch), a sugar (e.g., lactose, mannitol, sucrose, dextrose), a cellulosic material (e.g. microcrystalline cellulose), an acrylate (e.g. polymethylacrylate), calcium carbonate, magnesium oxide, talc, or mixtures thereof.

In other embodiments, pharmaceutically acceptable carriers for liquid formulations are aqueous or non-aqueous solutions, suspensions, emulsions or oils. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Examples of oils are those of animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, olive oil, sunflower oil, fish-liver oil, another marine oil, or a lipid from milk or eggs.

In another embodiment, parenteral vehicles (for subcutaneous, intravenous, intraarterial, or intramuscular injection) include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's and fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Examples are sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically acceptable adjuvants. In general, water, saline, aqueous dextrose and related sugar solutions, and glycols such as propylene glycols or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions. Examples of oils are those of animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, olive oil, sunflower oil, fish-liver oil, another marine oil, or a lipid from milk or eggs.

In another embodiment, the pharmaceutical compositions provided herein are controlled-release compositions, i.e. compositions in which the active ingredient is released over a period of time after administration. Controlled- or sustained-release compositions include formulation in lipophilic depots (e.g. fatty acids, waxes, oils). In another embodiment, the composition is an immediate-release composition, i.e. a composition in which all the active ingredient is released immediately after administration.

EXPERIMENTAL DETAILS SECTION

Example 1

Construction of LLO-HMW-MAA Constructs and *Listeria* Strains Expressing Same

LLO-HMW-MAA constructs were created as follows:

pGG-55, the precursor of the LLO-HMW-MAA constructs, was created from pAM401, a shuttle vector able to replicate in both gram-negative and gram-positive bacteria (Wirth R et al, J Bacteriol, 165: 831, 1986). pAM401 contains a gram-positive chloramphenicol resistance gene and gram negative tetracycline resistance determinant. In pGG-55, the hly promoter drives the expression of the first 441 AA of the hly gene product, (lacking the hemolytic C-terminus, having the sequence set forth in SEQ ID No: 3), which is joined by the XhoI site to the E7 gene, yielding a hly-E7 fusion gene that is transcribed and secreted as LLO-E7.

Generation of pGG-55: A fusion of a listeriolysin fragment to E7 ("LLO-E7") and the pluripotential transcription factor prfA were subcloned in pAM401 as follows: The DNA fragment encoding the first 420 AA of LLO and its promoter and upstream regulatory sequences was PCR amplified with LM genomic DNA used as a template and ligated into pUC19. PCR primers used were 5'-GGCCCGGGCCCCCTC-CTTTGAT-3' (SEQ ID No: 17) and 5'-GGTCTAGATCAT-AATTTACTTCATCC-3' (SEQ ID No: 18). E7 was amplified by PCR using the primers 5'-GGCTCGAGCATGGAGATA-CACC-3' (SEQ ID No: 19; XhoI site is underlined) and 5'-GGGGACTAGTTTATGGTTTCTGAGAACA-3' (SEQ ID No: 20; SpeI site is underlined) and ligated into pCR2.1 (Invitrogen, San Diego, Calif.). E7 was excised from pCR2.1 by XhoI/SpeI digestion and subsequently ligated as an in-frame translational fusion into pUC19-hly downstream of the hemolysin gene fragment. The fusion was then subcloned into the multilinker of pAM401. The prfA gene was then subcloned into the SalI site of the resulting plasmid, yielding pGG-55 (FIG. **1**).

pGG34A, B and C were created from pGG-55 as follows:

HMW-MAA fragments A, B, and C (encoding AA 360-554, 701-1130, and 2160-2258, respectively, FIG. **1**) have the following sequences:

HMW-MAA-A:

(SEQ ID No: 21)

Ttcaatggccagaggcgggggctgcgggaagctttgctgacgcgcaacatggcagccggctgcaggctggaggaggaggagtatgag

gacgatgcctatggacattatgaagctttctccaccctggcccctgaggcttggccagccatggagctgcctgagccatgcgtgcctgagccagggctg

cctcctgtctttgccaatttcacccagctgctgactatcagcccactggtggtggccgaggggggcacagcctggcttgagtggaggcatgtgcagccc

acgctggacctgatggaggctgagctgcgcaaatcccaggtgctgttcagcgtgacccgaggggcacgccatggcgagctcgagctggacatcccg

ggagcccaggcacgaaaaatgttcaccctcctggacgtggtgaaccgcaaggcccgcttcatccacgatggctctgaggacacctccgaccagctggt

gctggaggtgtcggtgacggctcgggtgcccatgccctcatgccttcggaggggccaaacatacctcctgcccatccaggtcaaccctgtcaatgaccc

-continued

accccac.

HMW-MAA-B:

(SEQ ID No: 22)

Gtccgcgtcactggggccctgcagttttggggagctgcagaaacacggggcaggtggggtggagggtgctgagtggtgggccacacagg

cgttccaccagcgggatgtggagcagggccgcgtgaggtacctgagcactgacccacagcaccacgcttacgacaccgtggagaacctggccctgg

aggtgcaggtgggccaggagatcctgagcaatctgtccttcccagtgaccatccagagagccactgtgtggatgctgcggctggagccactgcacact

cagaacacccagcaggagaccctcaccacagcccacctggaggccaccctggaggaggcaggcccaagccccccaaccttccattatgaggtggtt

caggctcccaggaaaggcaaccttcaactacagggcacaaggctgtcagatggccagggcttcacccaggatgacatacaggctggccgggtgacct

attggggccacagcacgtgcctcagaggcagtcgaggacaccttccgtttccgtgtcacagctccaccatatttctccccactctatacctttccccatcca

cattggtggtgacccagatgcgcctgtcctcaccaatgtcctcctcgtggtgcctgagggtggtgagggtgtcctctctgctgaccacctctttgtcaagag

tctcaacagtgccagctacctctatgaggtcatggagcggccccgccatgggaggttggcttggcgtgggacacaggacaagaccactatggtgacatcc

ttcaccaatgaagacctgttgcgtggccggctggtctaccagcatgatgactccgagaccacagaagatgatatcccatttgttgctacccgccagggcg

agagcagtggtgacatggcctgggaggaggtacggggtgtcttccgagtggccatccagcccgtgaatgaccacgcccctgtgcagaccatcagcc

ggatcttccatgtggcccggggtgggcggcggctgctgactacagacgacgtggccttcagcgatgctgactcgggctttgctgacgcccagctggtgc

ttacccgcaaggacctcctctttggcagtatcgtggccgtagatgagcccacgcggcccatctaccgcttcacccaggaggacctcaggaagaggcga

gtactgttcgtgcactcaggggctgaccgtggctggatccagctgcaggtgtccgacgggcaacaccaggccactgcgctgctggaggtgcaggcct

cggaaccctacctccgtgtggcc.

HMW-MAA-C:

(SEQ ID No: 23)

Gccactgagccttacaatgctgcccggccctacagcgtggccctgctcagtgtccccgaggccgcccggacggaagcagggaagccag

agagcagcacccccacaggcgagccaggccccatggcatccagccctgagcccgctgtggccaagggaggcttcctgagcttccttgaggccaaca

tgttcagcgtcatcatccccatgtgcctggtacttctgctcctggcgctcatcctgcccctgctcttctacctccgaaaacgcaacaagacgggcaagcat

gacgtccag.

The fragments were amplified using the following primers. The XhoI sites in the forward primers and XmaI sites (A and C) or SpeI site (B) in the reverse primers are underlined:

Fragment A:-forward primer

(SEQ ID No: 24)

TCCTCGAGGTCAATGGCCAGAGGCGGGGG.

Reverse:

(SEQ ID No: 25)

CCCGGGTTACTACTTATCGTCGTCATCCTTGTAATCGTGGGGTGGGTCAT TGAC.

Fragment B: forward:

(SEQ ID No: 26)

GCCTCGAGTTCCGCGTCACTGGGGCCCTG.

Reverse:

(SEQ ID No: 27)

ACTAGTTTACTACTTATCGTCGTCATCCTTGTAATCGGCCACACGGAGGT AGGGTTC.

Fragment C: Forward:

(SEQ ID No: 28)

TGCTCGAGGCCACTGAGCCTTACAATGCTGCC.

Reverse:

(SEQ ID No: 29)

CCCGGGTTACTACTTATCGTCGTCATCCTTGTAATCCTGGACGTCATGCT TGCCCG.

Fragments A-C were then subcloned into pGG-55, using the XhoI site at the end of the hly sequence and the XmaI or SpeI site following the gene.

A prfA negative strain of Listeria, XFL-7 (provided by Dr. Hao Shen, University of Pennsylvania), was then transformed with pGG34A, B and C, to select for the retention of the plasmids in vivo.

Example 2

LLO-HMW-MAA Constructs are Expressed in *Listeria*

Materials and Experimental Methods

Bacteria Cultivation and Harvesting

Recombinant *Listeria monocytogenes* (LM) expressing the HMW-MAA fragments A, B and C fused to LLO were grown overnight in BHI medium supplemented with streptomycin (250 ug/ml) and chloramphenicol (25 ug/ml). For induction of endogeneous LLO, bacteria were cultivated in the presence of 0.2% charcoal. Culture supernatants were cleared by centrifugation at 14000 rpm for 5 minutes, and 1.35 milliliters (ml) supernatant was mixed with 0.15 ml of 100% TCA for protein precipitation. After incubation on ice for 1 hour, the solution was spun for 10 minutes, 14000 rpm. The pellet was resuspended in 45 microliter (mcL) of 1×SDS-PAGE gel loading buffer, 5 mcL of 1 M DTT was added, and the sample was heated at 75° C. for 5 minutes. 5-10 mcL of protein was loaded into each well and run for 50 minutes at 200V using MOPS buffer.

After transfer to PVDF membranes, membranes were incubated with either a rabbit anti-PEST polyclonal antibody

(1:3000), which recognizes the PEST sequence in the LLO protein, or with the B3-19 monoclonal antibody, which recognizes the endogenous LLO only, then incubated with HRP-conjugated anti-rabbit antibody. Signals were detected with SuperSignal® West Pico Chemiluminescent Substrate (Pierce, Rockford, Ill.).

Results

To determine whether the LLO-HMW-MAA constructs could be expressed in *Listeria*, supernatant was harvested from LM strains transformed with the LLO-HMW-MAA A, B and C plasmids, and assayed for presence of the fusion proteins. All three strains produced fusion proteins of the expected sizes when probed with anti-PEST antibody (48 Kda for LLO, 75 Kda for HMW-MAA-A, 98 Kda for HMW-MAA-B, and 62 Kda for HMW-MAA-C; FIG. **2**A). Anti-LLO antibody revealed 58 Kda band for LLO in all three strains and controls (FIG. **2**B).

Thus, LLO-HMW-MAA constructs are expressed in *Listeria.*

Example 3

Listeria Strains Expressing LLO-HMW-MAA Constructs Infect and Grow Inside Cells

Materials and Experimental Methods

Cell Infection Assay

Murine macrophage-like J774 cells were infected at a MOI (multiplicity of infection) of 1. After a 1-hour incubation, gentamicin was added to kill extracellular *Listeria*, intracellular *Listeria* was recovered every 2 hours by lysing the J774 cells with water and plating serial dilutions of the lysate on BHI plates supplemented with streptomycin (250 micrograms (mcg)/ml) and chloramphenicol (25 mcg/ml). Recovered colonies were counted and used to determine the number of *Listeria* inside J774 cells.

Results

To determine the growth characteristics and virulence of *Listeria* strains expressing LLO-HMW-MAA constructs, the growth rate of *Listeria* strains from the previous Example in BHI media was measured. Each of the strains grew with kinetics very similar to wild-type (1043S) *Listeria* (FIG. **3**A). Next, J774 cells were incubated with the *Listeria* strains, and intracellular growth was measured. Intracellular growth was very similar to wild-type for each strain (FIG. **3**B).

Thus, *Listeria* strains expressing LLO-HMW-MAA constructs maintain their ability to grow in media, to infect cells, and to grow intracellularly.

Example 4

Vaccination with HMW-MAA-Expressing Lm Impedes B16F0-OVA Tumor Growth

Materials and Experimental Methods

Measurement of Tumor Growth

Tumors were measured every second day with calipers spanning the shortest and longest surface diameters. The mean of these two measurements was plotted as the mean tumor diameter in millimeters against various time points. Mice were sacrificed when the tumor diameter reached 20 mm. Tumor measurements for each time point are shown only for surviving mice.

Results

32 C57BL/6 mice (n=8 per group) were inoculated with 5×10^5 B16F0-Ova. On days 3, 10 and 17 the mice were immunized with one of3 constructs, Lm-OVA (10^6 cfu), Lm-LLO-OVA (10^8 cfu; positive control), and Lm-LLO-HMW-MAA-C (10^8 cfu). Despite the lack of expression of HMW-MAA by the tumor cells, Lm-LLO-HMW-MAA-C vaccination impeded tumor growth, significantly, but to a lesser extent than Lm-LLO-OVA (FIGS. **4**A-B). In an additional experiment, similar results were observed with all 3 Lm-LLO-HMW-MAA strains (2.5×10^7 cfu each of A and C; 1×10^8 cfu of B; FIG. **4**C).

A similar experiment was performed with RENCA cells. 40 BALB/c mice (n=8 per group) were inoculated with 2×10^5 RENCA tumor cells. On days 3, 10, and 17, the mice were immunized with one of four constructs, Lm-HMW-MAA-A, B, or C ((2.5×10^7 cfu each of A and C; 1×10^8 cfu of B), or GGE7 (Lm-LLO-E7; 1.0×10^8 cfu), or were left unvaccinated (naïve). All three Lm-LLO-HMW-MAA strains impeded tumor growth, with Lm-HMW-MAA-C exerting the strongest effect (FIGS. **4**D-**4**E).

Thus, vaccination with HMW-MAA-expressing Lm impedes the growth of tumors, even in the absence of expression of HMW-MAA by the tumor cells.

Example 5

Vaccination with HMW-MAA-Expressing Lm Impedes B16F10-HMW-MAA Tumor Growth Via Cd4+ and Cd8+ Cells

Materials and Experimental Methods

Engineering of B16 and B16F10 Murine Tumor Cell Lines to Express HMW-MAA

B16F10 cells were transfected with pcDNA3.1-HMW-MAA plasmid, containing the full-length HMW-MAA cDNA expressed under the control of the CMV promoter. Stably transfected cells were selected by resistance to G418 antibiotic, and clones were subsequently grown from single cells by limiting dilution. Selected clones were tested by flow cytometry for HMW-MAA expression using the HMW-MAA specific monoclonal antibody VT80.12. Based on the flow cytometry results, B16F10-HMW-MAA clone 7 was selected for future experiments (FIG. **5**).

CD4+ and CD8+ Depletion

32 C57BL/6 mice were inoculated with 2×10^5 B16F10-HMW-MAA/CMV7. On days 3, 10 and 17 the mice were immunized with Lm-HMW-MAA-C (2.5×10^7 cfu), except the control naïve group. For CD4 and CD8 depletions, 500 μg of GK1.5 and 2.43 were given i.p. on days 1, 2, 6 and 9, as well as the control antibody G1. For CD25 depletion, 500 μg of CP61 was given i.p. on days 0 and 2.

Immunization of HLA A2/K^b Transgenic Mice with Lm-HMW-MAA-B or Lm-HMW-MAA-C

HLA A2/K^b transgenic mice express a chimeric class I molecule composed of the α1 and α2 domains of the human A*0201 allele and the α3 domains of the mouse H-2K^b class I molecules. HLA-A2/K^b transgenic mice were immunized once with either 1.0×10^8 cfu of Lm-HMW-MAA-B or 2.5×10^7 cfu of Lm-HMW-MAA-C. 9 days later, splenocytes were stimulated in vitro with peptide B_1 (ILSNLSFPV; SEQ ID

NO: 43; corresponds to HMW-MAA$_{769-777}$), peptide B$_2$ (LLFGSIVAV; SEQ ID NO: 44; corresponds to HMW-MAA$_{1063-1071}$), or peptide C (LILPLLFYL; SEQ ID NO: 45; corresponds to HMW-MAA$_{2238-2246}$) for 5 hours in the presence of monensin. Cells were gated on CD8+-CD62L^{low} and IFN-γ intracellular staining was measured.

In a separate experiment, mice were immunized twice (day 0 and day 7) with either Lm-HMW-MAA-B or Lm-HMW-MAA-C and splenocytes harvested on day 14 for in vitro stimulation with fragment B1 or C of Lm-HMW-MAA. IFN-γ levels were measured using IFN-γ Elispot.

Results

C57BL/6 mice (n=8 per group) were inoculated with 2×10^5 B16F10-HMW-MAA/CMV7. On days 3, 10 and 17, mice were immunized with one of three constructs, Lm-HMW-MAA-A (2.5×10^7 cfu), Lm-HMW-MAA-B (1×10^8 cfu), Lm-HMW-MAA-C (2.5×10^7 cfu). The control group was vaccinated with Lm-GGE7 (1×10^8 cfu). All three Lm-HMW-MAA constructs exerted significant anti-tumor effects (FIG. **6**).

To determine the role of CD4+ and CD8+ cells in the anti-tumor effect of Lm-HMW-MAA, CD4+ or CD8+, cells were depleted in C57BL/6 mice who had been innoculated with B16F10-HMW-MAA/CMV7B and immunized on days 3, 10, and 17 with Lm-HMW-MAA-C (2.5×10^7 cfu). CD4+ or CD8+ depletion abrogated the efficacy of LM-HMW-MAA-C vaccine (FIG. **7**).

CD8+ T cells (2×10^6 cells per mouse) were purified from the spleens of mice from each treatment group, mixed with B16F10-HMW-MAA tumor cells (2×10^5 per mouse), and then subcutaneously injected in mice (8 per group). Mice were observed for 28 days and examined every 2 days for tumor growth. CD8+ T cells from Lm-HMW-MAA-C-vaccinated mice inhibited the growth of B 16F10 HMW-MAA tumors in vivo (FIG. **8**).

Mice that had been inoculated with 2×10^5 B 16F10-HMW-MAA/CMV7 and vaccinated with Lm-HMW-MAA-C as described above and remained tumor-free after 7 weeks were re-challenged with 2×10^5 B16F10-HMW-MAA cells 7 weeks after the first tumor injection. Vaccinated mice were protected against a second challenge with B16F10-HMW-MAA/CMV7 tumor cells (FIG. **9**).

Immunization of HLA-A2/K^b transgenic mice with Lm-HMW-MAA-B and Lm-HMW-MAA-C induces detectable immune responses against two characterized HMW-MAA HLA-A2 epitopes in fragments B and C both after one (FIG. **10**A) or two immunizations (FIG. **10**B).

HLA-A2/K^b and wild-type C57B1/6 mice were immunized once with Lm-HMW-MAA-B or Lm-HMW-MAA-C, and IFN-γ secretion by T cells stimulated with an HLA-A2 restricted peptide from fragment C was measured with IFN-γ Elispot. IFN-γ secretion was increased in Lm-HMW-MAA-C-immunized HLA-A2/K^b transgenic mice stimulated with Peptide C compared to unstimulated transgenic mice, compared to Peptide C-stimulated non-transgenic mice and compared to non-immunized transgenic and control mice (FIGS. **11**A and **11**B).

Thus, Lm-HMW-MAA constructs induce antigen-specific immune responses that impede tumor growth. In addition, the Lm-HMW-MAA constructs exhibit anti-tumor activity even against tumors not expressing HMW-MAA.

Example 6

Fusion oF E7 to LLO or ActA Enhances E7-Specific Immunity and Generates Tumor-Infiltrating E7-Specific CD8$^+$ Cells

Materials and Experimental Methods

Construction of Lm-actA-E7

Lm-actA-E7 was generated by introducing a plasmid vector pDD-1 constructed by modifying pDP-2028 into LM. pDD-1 comprises an expression cassette expressing a copy of the 310 bp hly promoter and the hly signal sequence (ss), which drives the expression and secretion of actA-E7; 1170 bp of the actA gene that comprises 4 PEST sequences (SEQ ID NO: 5) (the truncated ActA polypeptide consists of the first 390 AA of the molecule, SEQ ID NO: 4); the 300 bp HPV E7 gene; the 1019 bp prfA gene (controls expression of the virulence genes); and the CAT gene (chloramphenicol resistance gene) for selection of transformed bacteria clones.

pDD-1 was created from pDP2028 (encoding ALLO-NP), which was in turn created from pDP1659 as follows:

Construction of pDP1659: The DNA fragment encoding the first 420 AA of LLO and its promoter and upstream regulatory sequences was PCR amplified with LM genomic DNA used as a template and ligated into pUC19. PCR primers used were 5'-GGCCCGGGCCCCCTCCTTTGAT-3' (SEQ ID No: 30) and 5'-GGTCTAGATCATAATTTACTTCATCC-3' (SEQ ID No: 31). The DNA fragment encoding NP was similarly PCR amplified from linearized plasmid pAPR501 (obtained from Dr. Peter Palese, Mt. Sinai Medical School, New York) and subsequently ligated as an in-frame translational fusion into pUC19 downstream of the hemolysin gene fragment. PCR primers used were 5'-GGTCTAGAGAATTC-CAGCAAAAGCAG-3' (SEQ ID No: 32) and 5'-GGGTC-GACAAGGGTATTTTTCTTTAAT-3' (SEQ ID No: 33). The fusion was then subcloned into the EcoRV and SalI sites of pAM401. Plasmid pDP2028 was constructed by subcloning the prfA gene into the SalI site of pDP1659.

pDD-1 was created from pDP-2028 (Lm-LLO-NP) as follows:

The hly promoter (pHly) and gene fragment (441 AA) were PCR amplified from pGG55 using primer 5'-GGGGTCTA-GACCTCCTTTGATTAGTATATTC-3' (XbaI site is underlined; SEQ ID NO: 34) and primer 5'-ATCTTCGCTATCT-GTCGCCGCGGCGCGTGCTTCAGTTTGTTGCGC-'3 (Not I site is underlined. The first 18 nucleotides are the ActA gene overlap; SEQ ID NO: 35). The actA gene was PCR amplified from the LM 10403s wildtype genome using primer 5'-GCGCAACAAACTGAAGCAGCGGCCGCG-GCGACAGATAGCGAAGAT-3' (NotI site is underlined; SEQ ID NO: 36) and primer 5'-TGTAGGTGTATCTCCAT-GCTCGAGAGCTAGGCGATCAATTTC-3' (XhoI site is underlined; SEQ ID NO: 37). The E7 gene was PCR amplified from pGG55 using primer 5'-GGAATTGATCGC-CTAGCTCTCGAGCATGGAGATACACCTACA-3' (XhoI site is underlined; SEQ ID NO: 38) and primer 5'-AAACG-GATTTATTTAGATCCCGGGTTATG-GTTTCTGAGAACA-3' (XmaI site is underlined; SEQ ID NO: 39). The prfA gene was PCR amplified from the LM 10403s wild-type genome using primer 5'-TGTTCTCA-GAAACCATAACCCGGGATCTAAATAAATCCGTTT-3' (XmaI site is underlined; SEQ ID NO: 40) and primer 5'-GGGGGTCGACCAGCTCTTCTTGGTGAAG-3' (SalI site is underlined; SEQ ID NO: 41). The hly promoter-actA gene fusion (pHly-actA) was PCR generated and amplified

from purified pHly and actA DNA using the upstream pHly primer (SEQ ID NO: 34) and downstream actA primer (SEQ ID NO: 37).

The E7 gene fused to the prfA gene (E7-prfA) was PCR generated and amplified from purified E7 and prfA DNA using the upstream E7 primer (SEQ ID NO: 38) and downstream prfA gene primer (SEQ ID NO: 41).

The pHly-actA fusion product fused to the E7-prfA fusion product was PCR generated and amplified from purified fused pHly-actA and E7-prfA DNA products using the upstream pHly primer (SEQ ID NO: 34) and downstream prfA gene primer (SEQ ID NO: 41) and ligated into pCRII (Invitrogen, La Jolla, Calif.). Competent *E. coli* (TOP10'F, Invitrogen, La Jolla, Calif.) were transformed with pCRII-ActAE7. After lysis and isolation, the plasmid was screened by restriction analysis using BamHI (expected fragment sizes 770 and 6400 bp) and BstXI (expected fragment sizes 2800 and 3900) and screened by PCR using the above-described upstream pHly primer and downstream prfA gene primer.

The pHly-ActA-E7-PrfA DNA insert was excised from pCRII by XbaI/SalI digestion with and ligated into Xba I/Sal I digested pDP-2028. After transforming TOP10'F competent *E. coli* (Invitrogen, La Jolla, Calif.) with expression system pHly-ActA-E7, chloramphenicol resistant clones were screened by PCR analysis using the above-described upstream pHly primer and downstream prfA gene primer. A clone containing pHly-ActA-E7 was amplified, and midiprep DNA was isolated (Promega, Madison, Wis). XFL-7 was transformed with pHly-ActA-E7, and clones were selected for the retention of the plasmid in vivo. Clones were grown in brain heart infusion medium (Difco, Detroit, Mich.) with 20 mcg (microgram)/ml (milliliter) chloramphenicol at 37° C. Bacteria were frozen in aliquots at −80° C.

In vivo Experiments

500 mcL of MATRIGEL®, containing 100 mcL of phosphate buffered saline (PBS) with 2×10^5 TC-1 tumor cells, plus 400 mcL of Matrigel® (BD Biosciences, Franklin Lakes, N.J.) were implanted subcutaneously on the left flank of 12 C57BL/6 mice (n=3). Mice were immunized intraperitoneally on day 7, 14 and 21, and spleens and tumors were harvested on day 28. Tumor Matrigels were removed from the mice and incubated at 4° C. overnight in tubes containing 2 ml RP 10 medium on ice. Tumors were minced.with forceps, cut into 2 mm blocks, and incubated at 37° C. for 1 hour with 3 ml of enzyme mixture (0.2 mg/ml collagenase-P, 1 mg/ml DNAse-1 in PBS). The tissue suspension was filtered through nylon mesh and washed with 5% fetal bovine serum+0.05% of NaN_3 in PBS for tetramer and IFN-gamma staining.

Splenocytes and tumor cells were incubated with 1 micromole (mcm) E7 peptide for 5 hours in the presence of brefeldin A at 10^7 cells/ml. Cells were washed twice and incubated in 50 mcL of anti-mouse Fc receptor supernatant (2.4 G2) for 1 hour or overnight at 4° C. Cells were stained for surface molecules CD8 and CD62L, permeabilized, fixed using the permeabilization kit Golgi-stop® or Golgi-Plug® (Pharmingen, San Diego, Calif.), and stained for IFN-gamma. 500,000 events were acquired using two-laser flow cytometer FACS-Calibur and analyzed using Cellquest Software (Becton Dickinson, Franklin Lakes, N.J.). Percentages of IFN-gamma secreting cells within the activated ($CD62L^{low}$) $CD8^+$ T cells were calculated.

For tetramer staining, H-$2D^b$ tetramer was loaded with phycoerythrin (PE)-conjugated E7 peptide (RAHYNIVTF, SEQ ID NO: 42), stained at rt for 1 hour, and stained with anti-allophycocyanin (APC) conjugated MEL-14 (CD62L) and FITC-conjugated CD8β at 4° C. for 30 min. Cells were analyzed comparing tetramer$^+$ $CD8^+$ $CD62L^{low}$ cells in the spleen and in the tumor.

Results

To analyze the ability of LLO and ActA fusions to enhance antigen specific immunity, mice were implanted with TC-1 tumor cells and immunized with either Lm-LLO-E7 (1×10^7 CFU), Lm-E7 (1×10^6 CFU), or Lm-ActA-E7 (2×10^8 CFU), or were untreated (naïve). Tumors of mice from the Lm-LLO-E7 and Lm-ActA-E7 groups contained a higher percentage of IFN-gamma-secreting $CD8^+$ T cells (FIG. **12**) and tetramer-specific $CD8^+$ cells (FIG. **13**) than in mice administered Lm-E7 or naive mice. Thus, Lm-LLO-E7 and Lm-ActA-E7 are both efficacious at induction of tumor-infiltrating $CD8^+$ T cells and tumor regression. Accordingly, LLO and ActA fusions are effective in methods and compositions of the present invention.

Example 7

Fusion to a PEST-Like Sequence Enhances E7-Specific Immunity

Materials and Experimental Methods

Constructs

Lm-PEST-E7, a *Listeria* strain identical to Lm-LLO-E7, except that it contains only the promoter and the first 50 AA of the LLO, was constructed as follows:

The hly promoter and PEST regions were fused to the full-length E7 gene by splicing by overlap extension (SOE) PCR. The E7 gene and the hly-PEST gene fragment were amplified from the plasmid pGG-55, which contains the first 441 amino acids of LLO, and spliced together by conventional PCR techniques. pVS16.5, the hly-PEST-E7 fragment and the LM transcription factor prfA were subcloned into the plasmid pAM401. The resultant plasmid was used to transform XFL-7.

$Lm\text{-}E7_{epi}$ is a recombinant strain that secretes E7 without the PEST region or an LLO fragment. The plasmid used to transform this strain contains a gene fragment of the hly promoter and signal sequence fused to the E7 gene. This construct differs from the original Lm-E7, which expressed a single copy of the E7 gene integrated into the chromosome. $Lm\text{-}E7_{epi}$ is completely isogenic to Lm-LLO-E7 and Lm-PEST-E7, except for the form of the E7 antigen expressed.

Recombinant strains were grown in brain heart infusion (BHI) medium with chloramphenicol (20 mcg/mL). Bacteria were frozen in aliquots at −80° C.

Results

To test the effect on antigenicity of fusion to a PEST-like sequence, the LLO PEST-like sequence was fused to E7. Tumor regression studies were performed, as described for Example 1, in parallel with *Listeria* strain expressing LLO-E7 and E7 alone. Lm-LLO-E7 and Lm-PEST-E7 caused the regression ⅝ and ⅜ established tumors, respectively (FIG. **14**). By contrast, Lm-E7epi only caused tumor regression in ⅛ mice. A statistically significant difference in tumor sizes was observed between tumors treated with PEST-containing constructs (Lm-LLO-E7 or Lm-PEST-E7) and those treated with Lm-E7epi (Student's t test).

To compare the levels of E7-specific lymphocytes generated by the vaccines in the spleen, spleens were harvested on

day 21 and stained with antibodies to CD62L, CD8, and the E7/Db tetramer. Lm-E7$_{epi}$ induced low levels of E7 tetramer-positive activated CD8$^+$ T cells in the spleen, while Lm-PEST-E7 and Lm-LLO-E7 induced 5 and 15 times more cells, respectively (FIG. **15**A), a result that was reproducible over 3 separate experiments. Thus, fusion to PEST-like sequences increased induction of tetramer-positive splenocytes. The mean and SE of data obtained from the 3 experiments (FIG. **15**B) demonstrate the significant increase in tetramer-positive CD8$^+$ cells by Lm-LLO-E7 and Lm-PEST-E7 overLm-E7epi ($P<0.05$ by Student's t test). Similarly, the number of tumor-infiltrating antigen-specific CD8$^+$ T cells was higher in mice vaccinated with Lm-LLO-E7 and Lm-PEST-E7, reproducibly over 3 experiments (FIG. **16**A-B). Average values of tetramer-positive CD8$^+$ TILs were significantly higher for Lm-LLO-E7 than Lm-E7epi ($P<0.05$; Student's t test.

Thus, PEST-like sequences confer increased immunogenicity to antigens.

Example 8

Enhancement of Immunogenicity by Fusion of an Antigen to LLO Does Not Require a *Listeria* Vector

Materials and Experimental Methods

Construction of Vac-SigE7Lamp

The WR strain of vaccinia was used as the recipient, and the fusion gene was excised from the Listerial plasmid and inserted into pSC11 under the control of the p75 promoter. This vector was chosen because it is the transfer vector used for the vaccinia constructs Vac-SigE7Lamp and Vac-E7 and therefore allowed direct comparison with Vac-LLO-E7. In this way all 3 vaccinia recombinants were expressed under control of the same early/late compound promoter p7.5. In addition, SC11 allows the selection of recombinant viral plaques to TK selection and beta-galactosidase screening. FIG. **17** depicts the various vaccinia constructs used in these experiments. Vac-SigE7Lamp is a recombinant vaccinia virus that expressed the E7 protein fused between lysosomal associated membrane protein (LAMP-1) signal sequence and sequence from the cytoplasmic tail of LAMP-1.

The following modifications were made to allow expression of the gene product by vaccinia: (a) the T5XT sequence that prevents early transcription by vaccinia was removed from the 5' portion of the LLO-E7 sequence by PCR; and (b) an additional XmaI restriction site was introduced by PCR to allow the final insertion of LLO-E7 into SC11. Successful introduction of these changes (without loss of the original sequence that encodes for LLO-E7) was verified by sequencing. The resulting pSC1 1-E7 construct was used to transfect the TK-ve cell line CV1 that had been infected with the wild-type vaccinia strain, WR. Cell lysates obtained from this co-infection/transfection step contain vaccinia recombinants that were plaque-purified 3 times. Expression of the LLO-E7 fusion product by plaque-purified vaccinia was verified by Western blot using an antibody directed against the LLO protein sequence. Ability of Vac-LLO-E7 to produce CD8$^+$ T cells specific to LLO and E7 was determined using the LLO (91-99) and E7 (49-57) epitopes of Balb/c and C57/BL6 mice, respectively. Results were confirmed in a chromium release assay.

Results

To determine whether enhancement of immunogenicity by fusion of an antigen to LLO requires a *Listeria* vector, a vaccinia vector expressing E7 as a fusion protein with a non-hemolytic truncated form of LLO was constructed. Tumor rejection studies were performed with TC-1 as described in above Examples, but initiating treatment when the tumors were 3 mm in diameter (FIG. **18**). By day 76, 50% of the Vac-LLO-E7 treated mice were tumor free, while only 25% of the Vac-SigE7Lamp mice were tumor free. In other experiments, LLO-antigen fusions were shown to be more immunogenic than E7 peptide mixed with SBAS2 or unmethylated CpG oligonucleotides in a side-by-side comparison.

These results show that (a) LLO-antigen fusions are immunogenic not only in the context of *Listeria*, but also in other contexts; and (b) the immunogenicity of LLO-antigen fusions compares favorably with other vaccine approaches known to be efficacious.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 45

<210> SEQ ID NO 1
<211> LENGTH: 529
<212> TYPE: PRT
<213> ORGANISM: Listeria monocytogenes

<400> SEQUENCE: 1

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            20                  25                  30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
        35                  40                  45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Glu Ile Asp Lys Tyr
    50                  55                  60

Ile Gln Gly Leu Asp Tyr Asn Lys Asn Asn Val Leu Val Tyr His Gly
65                  70                  75                  80
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-continued

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Asp Ala Val Thr Asn Val Pro Pro Arg Lys Gly Tyr Lys Asp Gly Asn
                85                  90                  95

Glu Tyr Ile Val Val Glu Lys Lys Lys Lys Ser Ile Asn Gln Asn Asn
            100                 105                 110

Ala Asp Ile Gln Val Val Asn Ala Ile Ser Ser Leu Thr Tyr Pro Gly
        115                 120                 125

Ala Leu Val Lys Ala Asn Ser Glu Leu Val Glu Asn Gln Pro Asp Val
    130                 135                 140

Leu Pro Val Lys Arg Asp Ser Leu Thr Leu Ser Ile Asp Leu Pro Gly
145                 150                 155                 160

Met Thr Asn Gln Asp Asn Lys Ile Val Val Lys Asn Ala Thr Lys Ser
                165                 170                 175

Asn Val Asn Asn Ala Val Asn Thr Leu Val Glu Arg Trp Asn Glu Lys
            180                 185                 190

Tyr Ala Gln Ala Tyr Pro Asn Val Ser Ala Lys Ile Asp Tyr Asp Asp
        195                 200                 205

Glu Met Ala Tyr Ser Glu Ser Gln Leu Ile Ala Lys Phe Gly Thr Ala
    210                 215                 220

Phe Lys Ala Val Asn Asn Ser Leu Asn Val Asn Phe Gly Ala Ile Ser
225                 230                 235                 240

Glu Gly Lys Met Gln Glu Glu Val Ile Ser Phe Lys Gln Ile Tyr Tyr
                245                 250                 255

Asn Val Asn Val Asn Glu Pro Thr Arg Pro Ser Arg Phe Phe Gly Lys
            260                 265                 270

Ala Val Thr Lys Glu Gln Leu Gln Ala Leu Gly Val Asn Ala Glu Asn
        275                 280                 285

Pro Pro Ala Tyr Ile Ser Ser Val Ala Tyr Gly Arg Gln Val Tyr Leu
    290                 295                 300

Lys Leu Ser Thr Asn Ser His Ser Thr Lys Val Lys Ala Ala Phe Asp
305                 310                 315                 320

Ala Ala Val Ser Gly Lys Ser Val Ser Gly Asp Val Glu Leu Thr Asn
                325                 330                 335

Ile Ile Lys Asn Ser Ser Phe Lys Ala Val Ile Tyr Gly Gly Ser Ala
            340                 345                 350

Lys Asp Glu Val Gln Ile Ile Asp Gly Asn Leu Gly Asp Leu Arg Asp
        355                 360                 365

Ile Leu Lys Lys Gly Ala Thr Phe Asn Arg Glu Thr Pro Gly Val Pro
    370                 375                 380

Ile Ala Tyr Thr Thr Asn Phe Leu Lys Asp Asn Glu Leu Ala Val Ile
385                 390                 395                 400

Lys Asn Asn Ser Glu Tyr Ile Glu Thr Thr Ser Lys Ala Tyr Thr Asp
                405                 410                 415

Gly Lys Ile Asn Ile Asp His Ser Gly Gly Tyr Val Ala Gln Phe Asn
            420                 425                 430

Ile Ser Trp Asp Glu Val Asn Tyr Asp Pro Glu Gly Asn Glu Ile Val
        435                 440                 445

Gln His Lys Asn Trp Ser Glu Asn Asn Lys Ser Lys Leu Ala His Phe
    450                 455                 460

Thr Ser Ser Ile Tyr Leu Pro Gly Asn Ala Arg Asn Ile Asn Val Tyr
465                 470                 475                 480

Ala Lys Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Thr Val Ile
                485                 490                 495

Asp Asp Arg Asn Leu Pro Leu Val Lys Asn Arg Asn Ile Ser Ile Trp
            500                 505                 510
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Gly Thr Thr Leu Tyr Pro Lys Tyr Ser Asn Lys Val Asp Asn Pro Ile
        515                 520                 525

Glu


<210> SEQ ID NO 2
<211> LENGTH: 441
<212> TYPE: PRT
<213> ORGANISM: Listeria monocytogenes

<400> SEQUENCE: 2

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1               5                   10                  15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
            20                  25                  30

Glu Asn Ser Ile Ser Ser Val Ala Pro Pro Ala Ser Pro Pro Ala Ser
        35                  40                  45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Glu Ile Asp Lys Tyr
    50                  55                  60

Ile Gln Gly Leu Asp Tyr Asn Lys Asn Asn Val Leu Val Tyr His Gly
65                  70                  75                  80

Asp Ala Val Thr Asn Val Pro Pro Arg Lys Gly Tyr Lys Asp Gly Asn
                85                  90                  95

Glu Tyr Ile Val Val Glu Lys Lys Lys Lys Ser Ile Asn Gln Asn Asn
            100                 105                 110

Ala Asp Ile Gln Val Val Asn Ala Ile Ser Ser Leu Thr Tyr Pro Gly
        115                 120                 125

Ala Leu Val Lys Ala Asn Ser Glu Leu Val Glu Asn Gln Pro Asp Val
    130                 135                 140

Leu Pro Val Lys Arg Asp Ser Leu Thr Leu Ser Ile Asp Leu Pro Gly
145                 150                 155                 160

Met Thr Asn Gln Asp Asn Lys Ile Val Val Lys Asn Ala Thr Lys Ser
                165                 170                 175

Asn Val Asn Asn Ala Val Asn Thr Leu Val Glu Arg Trp Asn Glu Lys
            180                 185                 190

Tyr Ala Gln Ala Tyr Ser Asn Val Ser Ala Lys Ile Asp Tyr Asp Asp
        195                 200                 205

Glu Met Ala Tyr Ser Glu Ser Gln Leu Ile Ala Lys Phe Gly Thr Ala
    210                 215                 220

Phe Lys Ala Val Asn Asn Ser Leu Asn Val Asn Phe Gly Ala Ile Ser
225                 230                 235                 240

Glu Gly Lys Met Gln Glu Glu Val Ile Ser Phe Lys Gln Ile Tyr Tyr
                245                 250                 255

Asn Val Asn Val Asn Glu Pro Thr Arg Pro Ser Arg Phe Phe Gly Lys
            260                 265                 270

Ala Val Thr Lys Glu Gln Leu Gln Ala Leu Gly Val Asn Ala Glu Asn
        275                 280                 285

Pro Pro Ala Tyr Ile Ser Ser Val Ala Tyr Gly Arg Gln Val Tyr Leu
    290                 295                 300

Lys Leu Ser Thr Asn Ser His Ser Thr Lys Val Lys Ala Ala Phe Asp
305                 310                 315                 320

Ala Ala Val Ser Gly Lys Ser Val Ser Gly Asp Val Glu Leu Thr Asn
                325                 330                 335

Ile Ile Lys Asn Ser Ser Phe Lys Ala Val Ile Tyr Gly Gly Ser Ala
            340                 345                 350
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Lys Asp Glu Val Gln Ile Ile Asp Gly Asn Leu Gly Asp Leu Arg Asp
        355                 360                 365

Ile Leu Lys Lys Gly Ala Thr Phe Asn Arg Glu Thr Pro Gly Val Pro
    370                 375                 380

Ile Ala Tyr Thr Thr Asn Phe Leu Lys Asp Asn Glu Leu Ala Val Ile
385                 390                 395                 400

Lys Asn Asn Ser Glu Tyr Ile Glu Thr Thr Ser Lys Ala Tyr Thr Asp
                405                 410                 415

Gly Lys Ile Asn Ile Asp His Ser Gly Gly Tyr Val Ala Gln Phe Asn
            420                 425                 430

Ile Ser Trp Asp Glu Val Asn Tyr Asp
        435                 440


<210> SEQ ID NO 3
<211> LENGTH: 416
<212> TYPE: PRT
<213> ORGANISM: Listeria monocytogenes

<400> SEQUENCE: 3

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1               5                   10                  15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
            20                  25                  30

Glu Asn Ser Ile Ser Ser Val Ala Pro Pro Ala Ser Pro Pro Ala Ser
        35                  40                  45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Glu Ile Asp Lys Tyr
    50                  55                  60

Ile Gln Gly Leu Asp Tyr Asn Lys Asn Asn Val Leu Val Tyr His Gly
65                  70                  75                  80

Asp Ala Val Thr Asn Val Pro Pro Arg Lys Gly Tyr Lys Asp Gly Asn
                85                  90                  95

Glu Tyr Ile Val Val Glu Lys Lys Lys Lys Ser Ile Asn Gln Asn Asn
            100                 105                 110

Ala Asp Ile Gln Val Val Asn Ala Ile Ser Ser Leu Thr Tyr Pro Gly
        115                 120                 125

Ala Leu Val Lys Ala Asn Ser Glu Leu Val Glu Asn Gln Pro Asp Val
    130                 135                 140

Leu Pro Val Lys Arg Asp Ser Leu Thr Leu Ser Ile Asp Leu Pro Gly
145                 150                 155                 160

Met Thr Asn Gln Asp Asn Lys Ile Val Val Lys Asn Ala Thr Lys Ser
                165                 170                 175

Asn Val Asn Asn Ala Val Asn Thr Leu Val Glu Arg Trp Asn Glu Lys
            180                 185                 190

Tyr Ala Gln Ala Tyr Ser Asn Val Ser Ala Lys Ile Asp Tyr Asp Asp
        195                 200                 205

Glu Met Ala Tyr Ser Glu Ser Gln Leu Ile Ala Lys Phe Gly Thr Ala
    210                 215                 220

Phe Lys Ala Val Asn Asn Ser Leu Asn Val Asn Phe Gly Ala Ile Ser
225                 230                 235                 240

Glu Gly Lys Met Gln Glu Glu Val Ile Ser Phe Lys Gln Ile Tyr Tyr
                245                 250                 255

Asn Val Asn Val Asn Glu Pro Thr Arg Pro Ser Arg Phe Phe Gly Lys
            260                 265                 270

Ala Val Thr Lys Glu Gln Leu Gln Ala Leu Gly Val Asn Ala Glu Asn
        275                 280                 285
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```
Pro Pro Ala Tyr Ile Ser Ser Val Ala Tyr Gly Arg Gln Val Tyr Leu
    290                 295                 300

Lys Leu Ser Thr Asn Ser His Ser Thr Lys Val Lys Ala Ala Phe Asp
305                 310                 315                 320

Ala Ala Val Ser Gly Lys Ser Val Ser Gly Asp Val Glu Leu Thr Asn
                325                 330                 335

Ile Ile Lys Asn Ser Ser Phe Lys Ala Val Ile Tyr Gly Gly Ser Ala
            340                 345                 350

Lys Asp Glu Val Gln Ile Ile Asp Gly Asn Leu Gly Asp Leu Arg Asp
        355                 360                 365

Ile Leu Lys Lys Gly Ala Thr Phe Asn Arg Glu Thr Pro Gly Val Pro
    370                 375                 380

Ile Ala Tyr Thr Thr Asn Phe Leu Lys Asp Asn Glu Leu Ala Val Ile
385                 390                 395                 400

Lys Asn Asn Ser Glu Tyr Ile Glu Thr Thr Ser Lys Ala Tyr Thr Asp
                405                 410                 415


<210> SEQ ID NO 4
<211> LENGTH: 390
<212> TYPE: PRT
<213> ORGANISM: Listeria monocytogenes

<400> SEQUENCE: 4

Met Arg Ala Met Met Val Val Phe Ile Thr Ala Asn Cys Ile Thr Ile
1               5                   10                  15

Asn Pro Asp Ile Ile Phe Ala Ala Thr Asp Ser Glu Asp Ser Ser Leu
            20                  25                  30

Asn Thr Asp Glu Trp Glu Glu Glu Lys Thr Glu Glu Gln Pro Ser Glu
        35                  40                  45

Val Asn Thr Gly Pro Arg Tyr Glu Thr Ala Arg Glu Val Ser Ser Arg
    50                  55                  60

Asp Ile Lys Glu Leu Glu Lys Ser Asn Lys Val Arg Asn Thr Asn Lys
65                  70                  75                  80

Ala Asp Leu Ile Ala Met Leu Lys Glu Lys Ala Glu Lys Gly Pro Asn
                85                  90                  95

Ile Asn Asn Asn Asn Ser Glu Gln Thr Glu Asn Ala Ala Ile Asn Glu
            100                 105                 110

Glu Ala Ser Gly Ala Asp Arg Pro Ala Ile Gln Val Glu Arg Arg His
        115                 120                 125

Pro Gly Leu Pro Ser Asp Ser Ala Ala Glu Ile Lys Lys Arg Arg Lys
    130                 135                 140

Ala Ile Ala Ser Ser Asp Ser Glu Leu Glu Ser Leu Thr Tyr Pro Asp
145                 150                 155                 160

Lys Pro Thr Lys Val Asn Lys Lys Lys Val Ala Lys Glu Ser Val Ala
                165                 170                 175

Asp Ala Ser Glu Ser Asp Leu Asp Ser Ser Met Gln Ser Ala Asp Glu
            180                 185                 190

Ser Ser Pro Gln Pro Leu Lys Ala Asn Gln Gln Pro Phe Phe Pro Lys
        195                 200                 205

Val Phe Lys Lys Ile Lys Asp Ala Gly Lys Trp Val Arg Asp Lys Ile
    210                 215                 220

Asp Glu Asn Pro Glu Val Lys Lys Ala Ile Val Asp Lys Ser Ala Gly
225                 230                 235                 240

Leu Ile Asp Gln Leu Leu Thr Lys Lys Lys Ser Glu Glu Val Asn Ala
                245                 250                 255
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```
Ser Asp Phe Pro Pro Pro Pro Thr Asp Glu Glu Leu Arg Leu Ala Leu
            260                 265                 270

Pro Glu Thr Pro Met Leu Leu Gly Phe Asn Ala Pro Ala Thr Ser Glu
        275                 280                 285

Pro Ser Ser Phe Glu Phe Pro Pro Pro Pro Thr Asp Glu Glu Leu Arg
    290                 295                 300

Leu Ala Leu Pro Glu Thr Pro Met Leu Leu Gly Phe Asn Ala Pro Ala
305                 310                 315                 320

Thr Ser Glu Pro Ser Ser Phe Glu Phe Pro Pro Pro Pro Thr Glu Asp
                325                 330                 335

Glu Leu Glu Ile Ile Arg Glu Thr Ala Ser Ser Leu Asp Ser Ser Phe
            340                 345                 350

Thr Arg Gly Asp Leu Ala Ser Leu Arg Asn Ala Ile Asn Arg His Ser
        355                 360                 365

Gln Asn Phe Ser Asp Phe Pro Pro Ile Pro Thr Glu Glu Glu Leu Asn
    370                 375                 380

Gly Arg Gly Gly Arg Pro
385                 390
```

<210> SEQ ID NO 5
<211> LENGTH: 1170
<212> TYPE: DNA
<213> ORGANISM: Listeria monocytogenes

<400> SEQUENCE: 5

```
atgcgtgcga tgatggtggt tttcattact gccaattgca ttacgattaa ccccgacata     60

atatttgcag cgacagatag cgaagattct agtctaaaca cagatgaatg ggaagaagaa    120

aaaacagaag agcaaccaag cgaggtaaat acgggaccaa gatacgaaac tgcacgtgaa    180

gtaagttcac gtgatattaa agaactagaa aaatcgaata aagtgagaaa tacgaacaaa    240

gcagacctaa tagcaatgtt gaaagaaaaa gcagaaaaag gtccaaatat caataataac    300

aacagtgaac aaactgagaa tgcggctata aatgaagagg cttcaggagc cgaccgacca    360

gctatacaag tggagcgtcg tcatccagga ttgccatcgg atagcgcagc ggaaattaaa    420

aaaagaagga aagccatagc atcatcggat agtgagcttg aaagccttac ttatccggat    480

aaaccaacaa aagtaaataa gaaaaaagtg gcgaaagagt cagttgcgga tgcttctgaa    540

agtgacttag attctagcat gcagtcagca gatgagtctt caccacaacc tttaaaagca    600

aaccaacaac catttttccc taaagtattt aaaaaaataa aagatgcggg gaaatgggta    660

cgtgataaaa tcgacgaaaa tcctgaagta aagaaagcga ttgttgataa aagtgcaggg    720

ttaattgacc aattattaac caaaaagaaa agtgaagagg taaatgcttc ggacttcccg    780

ccaccaccta cggatgaaga gttaagactt gctttgccag agacaccaat gcttcttggt    840

tttaatgctc ctgctacatc agaaccgagc tcattcgaat ttccaccacc acctacggat    900

gaagagttaa gacttgcttt gccagagacg ccaatgcttc ttggttttaa tgctcctgct    960

acatcggaac cgagctcgtt cgaatttcca ccgcctccaa cagaagatga actagaaatc   1020

atccgggaaa cagcatcctc gctagattct agttttacaa gaggggattt agctagtttg   1080

agaaatgcta ttaatcgcca tagtcaaaat ttctctgatt tcccaccaat cccaacagaa   1140

gaagagttga acgggagagg cggtagacca                                    1170
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<210> SEQ ID NO 6
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Listeria monocytogenes

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<400> SEQUENCE: 6

Lys Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala
1 5 10 15

Ser Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Glu Ile Asp Lys
20 25 30

<210> SEQ ID NO 7
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Listeria monocytogenes

<400> SEQUENCE: 7

Lys Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala
1 5 10 15

Ser Pro Lys

<210> SEQ ID NO 8
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Listeria monocytogenes

<400> SEQUENCE: 8

Lys Thr Glu Glu Gln Pro Ser Glu Val Asn Thr Gly Pro Arg
1 5 10

<210> SEQ ID NO 9
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Listeria monocytogenes

<400> SEQUENCE: 9

Lys Ala Ser Val Thr Asp Thr Ser Glu Gly Asp Leu Asp Ser Ser Met
1 5 10 15

Gln Ser Ala Asp Glu Ser Thr Pro Gln Pro Leu Lys
20 25

<210> SEQ ID NO 10
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Listeria monocytogenes

<400> SEQUENCE: 10

Lys Asn Glu Glu Val Asn Ala Ser Asp Phe Pro Pro Pro Pro Thr Asp
1 5 10 15

Glu Glu Leu Arg
20

<210> SEQ ID NO 11
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Listeria monocytogenes

<400> SEQUENCE: 11

Arg Gly Gly Ile Pro Thr Ser Glu Glu Phe Ser Ser Leu Asn Ser Gly
1 5 10 15

Asp Phe Thr Asp Asp Glu Asn Ser Glu Thr Thr Glu Glu Glu Ile Asp
20 25 30

Arg

<210> SEQ ID NO 12
<211> LENGTH: 19

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<212> TYPE: PRT
<213> ORGANISM: Listeria seeligeri

<400> SEQUENCE: 12

Arg Ser Glu Val Thr Ile Ser Pro Ala Glu Thr Pro Glu Ser Pro Pro
1 5 10 15

Ala Thr Pro

<210> SEQ ID NO 13
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Streptococcus pyogenes

<400> SEQUENCE: 13

Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu Gln Pro
1 5 10 15

Lys

<210> SEQ ID NO 14
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Streptococcus equisimilis

<400> SEQUENCE: 14

Lys Gln Asn Thr Ala Asn Thr Glu Thr Thr Thr Thr Asn Glu Gln Pro
1 5 10 15

Lys

<210> SEQ ID NO 15
<211> LENGTH: 2322
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

Met Gln Ser Gly Arg Gly Pro Pro Leu Pro Ala Pro Gly Leu Ala Leu
1 5 10 15

Ala Leu Thr Leu Thr Met Leu Ala Arg Leu Ala Ser Ala Ala Ser Phe
20 25 30

Phe Gly Glu Asn His Leu Glu Val Pro Val Ala Thr Ala Leu Thr Asp
35 40 45

Ile Asp Leu Gln Leu Gln Phe Ser Thr Ser Gln Pro Glu Ala Leu Leu
50 55 60

Leu Leu Ala Ala Gly Pro Ala Asp His Leu Leu Leu Gln Leu Tyr Ser
65 70 75 80

Gly Arg Leu Gln Val Arg Leu Val Leu Gly Gln Glu Glu Leu Arg Leu
85 90 95

Gln Thr Pro Ala Glu Thr Leu Leu Ser Asp Ser Ile Pro His Thr Val
100 105 110

Val Leu Thr Val Val Glu Gly Trp Ala Thr Leu Ser Val Asp Gly Phe
115 120 125

Leu Asn Ala Ser Ser Ala Val Pro Gly Ala Pro Leu Glu Val Pro Tyr
130 135 140

Gly Leu Phe Val Gly Gly Thr Gly Thr Leu Gly Leu Pro Tyr Leu Arg
145 150 155 160

Gly Thr Ser Arg Pro Leu Arg Gly Cys Leu His Ala Ala Thr Leu Asn
165 170 175

Gly Arg Ser Leu Leu Arg Pro Leu Thr Pro Asp Val His Glu Gly Cys
180 185 190

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```
Ala Glu Glu Phe Ser Ala Ser Asp Asp Val Ala Leu Gly Phe Ser Gly
        195                 200                 205

Pro His Ser Leu Ala Ala Phe Pro Ala Trp Gly Thr Gln Asp Glu Gly
    210                 215                 220

Thr Leu Glu Phe Thr Leu Thr Thr Gln Ser Arg Gln Ala Pro Leu Ala
225                 230                 235                 240

Phe Gln Ala Gly Gly Arg Arg Gly Asp Phe Ile Tyr Val Asp Ile Phe
                245                 250                 255

Glu Gly His Leu Arg Ala Val Val Glu Lys Gly Gln Gly Thr Val Leu
            260                 265                 270

Leu His Asn Ser Val Pro Val Ala Asp Gly Gln Pro His Glu Val Ser
        275                 280                 285

Val His Ile Asn Ala His Arg Leu Glu Ile Ser Val Asp Gln Tyr Pro
    290                 295                 300

Thr His Thr Ser Asn Arg Gly Val Leu Ser Tyr Leu Glu Pro Arg Gly
305                 310                 315                 320

Ser Leu Leu Leu Gly Gly Leu Asp Ala Glu Ala Ser Arg His Leu Gln
                325                 330                 335

Glu His Arg Leu Gly Leu Thr Pro Glu Ala Thr Asn Ala Ser Leu Leu
            340                 345                 350

Gly Cys Met Glu Asp Leu Ser Val Asn Gly Gln Arg Arg Gly Leu Arg
        355                 360                 365

Glu Ala Leu Leu Thr Arg Asn Met Ala Ala Gly Cys Arg Leu Glu Glu
    370                 375                 380

Glu Glu Tyr Glu Asp Asp Ala Tyr Gly His Tyr Glu Ala Phe Ser Thr
385                 390                 395                 400

Leu Ala Pro Glu Ala Trp Pro Ala Met Glu Leu Pro Glu Pro Cys Val
                405                 410                 415

Pro Glu Pro Gly Leu Pro Pro Val Phe Ala Asn Phe Thr Gln Leu Leu
            420                 425                 430

Thr Ile Ser Pro Leu Val Val Ala Glu Gly Gly Thr Ala Trp Leu Glu
        435                 440                 445

Trp Arg His Val Gln Pro Thr Leu Asp Leu Met Glu Ala Glu Leu Arg
    450                 455                 460

Lys Ser Gln Val Leu Phe Ser Val Thr Arg Gly Ala Arg His Gly Glu
465                 470                 475                 480

Leu Glu Leu Asp Ile Pro Gly Ala Gln Ala Arg Lys Met Phe Thr Leu
                485                 490                 495

Leu Asp Val Val Asn Arg Lys Ala Arg Phe Ile His Asp Gly Ser Glu
            500                 505                 510

Asp Thr Ser Asp Gln Leu Val Leu Glu Val Ser Val Thr Ala Arg Val
        515                 520                 525

Pro Met Pro Ser Cys Leu Arg Arg Gly Gln Thr Tyr Leu Leu Pro Ile
    530                 535                 540

Gln Val Asn Pro Val Asn Asp Pro Pro His Ile Ile Phe Pro His Gly
545                 550                 555                 560

Ser Leu Met Val Ile Leu Glu His Thr Gln Lys Pro Leu Gly Pro Glu
                565                 570                 575

Val Phe Gln Ala Tyr Asp Pro Asp Ser Ala Cys Glu Gly Leu Thr Phe
            580                 585                 590

Gln Val Leu Gly Thr Ser Ser Gly Leu Pro Val Glu Arg Arg Asp Gln
        595                 600                 605

Pro Gly Glu Pro Ala Thr Glu Phe Ser Cys Arg Glu Leu Glu Ala Gly
    610                 615                 620
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Ser Leu Val Tyr Val His Arg Gly Gly Pro Ala Gln Asp Leu Thr Phe
625                 630                 635                 640

Arg Val Ser Asp Gly Leu Gln Ala Ser Pro Pro Ala Thr Leu Lys Val
                645                 650                 655

Val Ala Ile Arg Pro Ala Ile Gln Ile His Arg Ser Thr Gly Leu Arg
            660                 665                 670

Leu Ala Gln Gly Ser Ala Met Pro Ile Leu Pro Ala Asn Leu Ser Val
        675                 680                 685

Glu Thr Asn Ala Val Gly Gln Asp Val Ser Val Leu Phe Arg Val Thr
    690                 695                 700

Gly Ala Leu Gln Phe Gly Glu Leu Gln Lys Gln Gly Ala Gly Gly Val
705                 710                 715                 720

Glu Gly Ala Glu Trp Trp Ala Thr Gln Ala Phe His Gln Arg Asp Val
                725                 730                 735

Glu Gln Gly Arg Val Arg Tyr Leu Ser Thr Asp Pro Gln His His Ala
            740                 745                 750

Tyr Asp Thr Val Glu Asn Leu Ala Leu Glu Val Gln Val Gly Gln Glu
        755                 760                 765

Ile Leu Ser Asn Leu Ser Phe Pro Val Thr Ile Gln Arg Ala Thr Val
    770                 775                 780

Trp Met Leu Arg Leu Glu Pro Leu His Thr Gln Asn Thr Gln Gln Glu
785                 790                 795                 800

Thr Leu Thr Thr Ala His Leu Glu Ala Thr Leu Glu Glu Ala Gly Pro
                805                 810                 815

Ser Pro Pro Thr Phe His Tyr Glu Val Val Gln Ala Pro Arg Lys Gly
            820                 825                 830

Asn Leu Gln Leu Gln Gly Thr Arg Leu Ser Asp Gly Gln Gly Phe Thr
        835                 840                 845

Gln Asp Asp Ile Gln Ala Gly Arg Val Thr Tyr Gly Ala Thr Ala Arg
    850                 855                 860

Ala Ser Glu Ala Val Glu Asp Thr Phe Arg Phe Arg Val Thr Ala Pro
865                 870                 875                 880

Pro Tyr Phe Ser Pro Leu Tyr Thr Phe Pro Ile His Ile Gly Gly Asp
                885                 890                 895

Pro Asp Ala Pro Val Leu Thr Asn Val Leu Leu Val Val Pro Glu Gly
            900                 905                 910

Gly Glu Gly Val Leu Ser Ala Asp His Leu Phe Val Lys Ser Leu Asn
        915                 920                 925

Ser Ala Ser Tyr Leu Tyr Glu Val Met Glu Arg Pro Arg His Gly Arg
    930                 935                 940

Leu Ala Trp Arg Gly Thr Gln Asp Lys Thr Thr Met Val Thr Ser Phe
945                 950                 955                 960

Thr Asn Glu Asp Leu Leu Arg Gly Arg Leu Val Tyr Gln His Asp Asp
                965                 970                 975

Ser Glu Thr Thr Glu Asp Asp Ile Pro Phe Val Ala Thr Arg Gln Gly
            980                 985                 990

Glu Ser Ser Gly Asp Met Ala Trp  Glu Glu Val Arg Gly  Val Phe Arg
        995                 1000                1005

Val Ala  Ile Gln Pro Val Asn  Asp His Ala Pro Val  Gln Thr Ile
    1010                1015                1020

Ser Arg  Ile Phe His Val Ala  Arg Gly Gly Arg Arg  Leu Leu Thr
    1025                1030                1035

Thr Asp  Asp Val Ala Phe Ser  Asp Ala Asp Ser Gly  Phe Ala Asp
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    1040                1045                1050

Ala Gln  Leu Val Leu Thr Arg  Lys Asp Leu Leu Phe  Gly Ser Ile
    1055                1060                1065

Val Ala  Val Asp Glu Pro Thr  Arg Pro Ile Tyr Arg  Phe Thr Gln
    1070                1075                1080

Glu Asp  Leu Arg Lys Arg Arg  Val Leu Phe Val His  Ser Gly Ala
    1085                1090                1095

Asp Arg  Gly Trp Ile Gln Leu  Gln Val Ser Asp Gly  Gln His Gln
    1100                1105                1110

Ala Thr  Ala Leu Leu Glu Val  Gln Ala Ser Glu Pro  Tyr Leu Arg
    1115                1120                1125

Val Ala  Asn Gly Ser Ser Leu  Val Val Pro Gln Gly  Gly Gln Gly
    1130                1135                1140

Thr Ile  Asp Thr Ala Val Leu  His Leu Asp Thr Asn  Leu Asp Ile
    1145                1150                1155

Arg Ser  Gly Asp Glu Val His  Tyr His Val Thr Ala  Gly Pro Arg
    1160                1165                1170

Trp Gly  Gln Leu Val Arg Ala  Gly Gln Pro Ala Thr  Ala Phe Ser
    1175                1180                1185

Gln Gln  Asp Leu Leu Asp Gly  Ala Val Leu Tyr Ser  His Asn Gly
    1190                1195                1200

Ser Leu  Ser Pro Arg Asp Thr  Met Ala Phe Ser Val  Glu Ala Gly
    1205                1210                1215

Pro Val  His Thr Asp Ala Thr  Leu Gln Val Thr Ile  Ala Leu Glu
    1220                1225                1230

Gly Pro  Leu Ala Pro Leu Lys  Leu Val Arg His Lys  Lys Ile Tyr
    1235                1240                1245

Val Phe  Gln Gly Glu Ala Ala  Glu Ile Arg Arg Asp  Gln Leu Glu
    1250                1255                1260

Ala Ala  Gln Glu Ala Val Pro  Pro Ala Asp Ile Val  Phe Ser Val
    1265                1270                1275

Lys Ser  Pro Pro Ser Ala Gly  Tyr Leu Val Met Val  Ser Arg Gly
    1280                1285                1290

Ala Leu  Ala Asp Glu Pro Pro  Ser Leu Asp Pro Val  Gln Ser Phe
    1295                1300                1305

Ser Gln  Glu Ala Val Asp Thr  Gly Arg Val Leu Tyr  Leu His Ser
    1310                1315                1320

Arg Pro  Glu Ala Trp Ser Asp  Ala Phe Ser Leu Asp  Val Ala Ser
    1325                1330                1335

Gly Leu  Gly Ala Pro Leu Glu  Gly Val Leu Val Glu  Leu Glu Val
    1340                1345                1350

Leu Pro  Ala Ala Ile Pro Leu  Glu Ala Gln Asn Phe  Ser Val Pro
    1355                1360                1365

Glu Gly  Gly Ser Leu Thr Leu  Ala Pro Pro Leu Leu  Arg Val Ser
    1370                1375                1380

Gly Pro  Tyr Phe Pro Thr Leu  Leu Gly Leu Ser Leu  Gln Val Leu
    1385                1390                1395

Glu Pro  Pro Gln His Gly Ala  Leu Gln Lys Glu Asp  Gly Pro Gln
    1400                1405                1410

Ala Arg  Thr Leu Ser Ala Phe  Ser Trp Arg Met Val  Glu Glu Gln
    1415                1420                1425

Leu Ile  Arg Tyr Val His Asp  Gly Ser Glu Thr Leu  Thr Asp Ser
    1430                1435                1440
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Phe Val  Leu Met Ala Asn Ala  Ser Glu Met Asp Arg  Gln Ser His
    1445                1450                1455

Pro Val  Ala Phe Thr Val Thr  Val Leu Pro Val Asn  Asp Gln Pro
    1460                1465                1470

Pro Ile  Leu Thr Thr Asn Thr  Gly Leu Gln Met Trp  Glu Gly Ala
    1475                1480                1485

Thr Ala  Pro Ile Pro Ala Glu  Ala Leu Arg Ser Thr  Asp Gly Asp
    1490                1495                1500

Ser Gly  Ser Glu Asp Leu Val  Tyr Thr Ile Glu Gln  Pro Ser Asn
    1505                1510                1515

Gly Arg  Val Val Leu Arg Gly  Ala Pro Gly Thr Glu  Val Arg Ser
    1520                1525                1530

Phe Thr  Gln Ala Gln Leu Asp  Gly Gly Leu Val Leu  Phe Ser His
    1535                1540                1545

Arg Gly  Thr Leu Asp Gly Gly  Phe Arg Phe Arg Leu  Ser Asp Gly
    1550                1555                1560

Glu His  Thr Ser Pro Gly His  Phe Phe Arg Val Thr  Ala Gln Lys
    1565                1570                1575

Gln Val  Leu Leu Ser Leu Lys  Gly Ser Gln Thr Leu  Thr Val Cys
    1580                1585                1590

Pro Gly  Ser Val Gln Pro Leu  Ser Ser Gln Thr Leu  Arg Ala Ser
    1595                1600                1605

Ser Ser  Ala Gly Thr Asp Pro  Gln Leu Leu Leu Tyr  Arg Val Val
    1610                1615                1620

Arg Gly  Pro Gln Leu Gly Arg  Leu Phe His Ala Gln  Gln Asp Ser
    1625                1630                1635

Thr Gly  Glu Ala Leu Val Asn  Phe Thr Gln Ala Glu  Val Tyr Ala
    1640                1645                1650

Gly Asn  Ile Leu Tyr Glu His  Glu Met Pro Pro Glu  Pro Phe Trp
    1655                1660                1665

Glu Ala  His Asp Thr Leu Glu  Leu Gln Leu Ser Ser  Pro Pro Ala
    1670                1675                1680

Arg Asp  Val Ala Ala Thr Leu  Ala Val Ala Val Ser  Phe Glu Ala
    1685                1690                1695

Ala Cys  Pro Gln Arg Pro Ser  His Leu Trp Lys Asn  Lys Gly Leu
    1700                1705                1710

Trp Val  Pro Glu Gly Gln Arg  Ala Arg Ile Thr Val  Ala Ala Leu
    1715                1720                1725

Asp Ala  Ser Asn Leu Leu Ala  Ser Val Pro Ser Pro  Gln Arg Ser
    1730                1735                1740

Glu His  Asp Val Leu Phe Gln  Val Thr Gln Phe Pro  Ser Arg Gly
    1745                1750                1755

Gln Leu  Leu Val Ser Glu Glu  Pro Leu His Ala Gly  Gln Pro His
    1760                1765                1770

Phe Leu  Gln Ser Gln Leu Ala  Ala Gly Gln Leu Val  Tyr Ala His
    1775                1780                1785

Gly Gly  Gly Gly Thr Gln Gln  Asp Gly Phe His Phe  Arg Ala His
    1790                1795                1800

Leu Gln  Gly Pro Ala Gly Ala  Ser Val Ala Gly Pro  Gln Thr Ser
    1805                1810                1815

Glu Ala  Phe Ala Ile Thr Val  Arg Asp Val Asn Glu  Arg Pro Pro
    1820                1825                1830

Gln Pro  Gln Ala Ser Val Pro  Leu Arg Leu Thr Arg  Gly Ser Arg
    1835                1840                1845
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Ala Pro  Ile Ser Arg Ala Gln  Leu Ser Val Val Asp  Pro Asp Ser
    1850                1855                1860

Ala Pro  Gly Glu Ile Glu Tyr  Glu Val Gln Arg Ala  Pro His Asn
    1865                1870                1875

Gly Phe  Leu Ser Leu Val Gly  Gly Gly Leu Gly Pro  Val Thr Arg
    1880                1885                1890

Phe Thr  Gln Ala Asp Val Asp  Ser Gly Arg Leu Ala  Phe Val Ala
    1895                1900                1905

Asn Gly  Ser Ser Val Ala Gly  Ile Phe Gln Leu Ser  Met Ser Asp
    1910                1915                1920

Gly Ala  Ser Pro Pro Leu Pro  Met Ser Leu Ala Val  Asp Ile Leu
    1925                1930                1935

Pro Ser  Ala Ile Glu Val Gln  Leu Arg Ala Pro Leu  Glu Val Pro
    1940                1945                1950

Gln Ala  Leu Gly Arg Ser Ser  Leu Ser Gln Gln Gln  Leu Arg Val
    1955                1960                1965

Val Ser  Asp Arg Glu Glu Pro  Glu Ala Ala Tyr Arg  Leu Ile Gln
    1970                1975                1980

Gly Pro  Gln Tyr Gly His Leu  Leu Val Gly Gly Arg  Pro Thr Ser
    1985                1990                1995

Ala Phe  Ser Gln Phe Gln Ile  Asp Gln Gly Glu Val  Val Phe Ala
    2000                2005                2010

Phe Thr  Asn Phe Ser Ser Ser  His Asp His Phe Arg  Val Leu Ala
    2015                2020                2025

Leu Ala  Arg Gly Val Asn Ala  Ser Ala Val Val Asn  Val Thr Val
    2030                2035                2040

Arg Ala  Leu Leu His Val Trp  Ala Gly Gly Pro Trp  Pro Gln Gly
    2045                2050                2055

Ala Thr  Leu Arg Leu Asp Pro  Thr Val Leu Asp Ala  Gly Glu Leu
    2060                2065                2070

Ala Asn  Arg Thr Gly Ser Val  Pro Arg Phe Arg Leu  Leu Glu Gly
    2075                2080                2085

Pro Arg  His Gly Arg Val Val  Arg Val Pro Arg Ala  Arg Thr Glu
    2090                2095                2100

Pro Gly  Gly Ser Gln Leu Val  Glu Gln Phe Thr Gln  Gln Asp Leu
    2105                2110                2115

Glu Asp  Gly Arg Leu Gly Leu  Glu Val Gly Arg Pro  Glu Gly Arg
    2120                2125                2130

Ala Pro  Gly Pro Ala Gly Asp  Ser Leu Thr Leu Glu  Leu Trp Ala
    2135                2140                2145

Gln Gly  Val Pro Pro Ala Val  Ala Ser Leu Asp Phe  Ala Thr Glu
    2150                2155                2160

Pro Tyr  Asn Ala Ala Arg Pro  Tyr Ser Val Ala Leu  Leu Ser Val
    2165                2170                2175

Pro Glu  Ala Ala Arg Thr Glu  Ala Gly Lys Pro Glu  Ser Ser Thr
    2180                2185                2190

Pro Thr  Gly Glu Pro Gly Pro  Met Ala Ser Ser Pro  Glu Pro Ala
    2195                2200                2205

Val Ala  Lys Gly Gly Phe Leu  Ser Phe Leu Glu Ala  Asn Met Phe
    2210                2215                2220

Ser Val  Ile Ile Pro Met Cys  Leu Val Leu Leu Leu  Leu Ala Leu
    2225                2230                2235

Ile Leu  Pro Leu Leu Phe Tyr  Leu Arg Lys Arg Asn  Lys Thr Gly
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    2240                2245                2250

Lys His  Asp Val Gln Val Leu  Thr Ala Lys Pro Arg  Asn Gly Leu
    2255                2260                2265

Ala Gly  Asp Thr Glu Thr Phe  Arg Lys Val Glu Pro  Gly Gln Ala
    2270                2275                2280

Ile Pro  Leu Thr Ala Val Pro  Gly Gln Gly Pro Pro  Pro Gly Gly
    2285                2290                2295

Gln Pro  Asp Pro Glu Leu Leu  Gln Phe Cys Arg Thr  Pro Asn Pro
    2300                2305                2310

Ala Leu  Lys Asn Gly Gln Tyr  Trp Val
    2315                2320
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<210> SEQ ID NO 16
<211> LENGTH: 7011
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

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cctgtggcca cggctctgac cgacatagac ctgcagctgc agttctccac gtcccagccc    180

gaagccctcc ttctcctggc agcaggccca gctgaccacc tcctgctgca gctctactct    240

ggacgcctgc aggtcagact tgttctgggc caggaggagc tgaggctgca gactccagca    300

gagacgctgc tgagtgactc catcccccac actgtggtgc tgactgtcgt agagggctgg    360

gccacgttgt cagtcgatgg gtttctgaac gcctcctcag cagtcccagg agccccccta    420

gaggtcccct atgggctctt tgttgggggc actgggaccc ttggcctgcc ctacctgagg    480

ggaaccagcc gacccctgag gggttgcctc catgcagcca ccctcaatgg ccgcagcctc    540

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ctcctgccca tccaggtcaa ccctgtcaat gacccacccc acatcatctt cccacatggc   1680
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ccggccatac agatccaccg cagcacaggg ttgcgactgg cccaaggctc tgccatgccc 2040

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<210> SEQ ID NO 17
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 17

ggcccgggcc ccctcctttg at 22

<210> SEQ ID NO 18
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 18

ggtctagatc ataatttact tcatcc 26

<210> SEQ ID NO 19
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 19

ggctcgagca tggagataca cc 22

<210> SEQ ID NO 20
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 20

ggggactagt ttatggtttc tgagaaca 28

<210> SEQ ID NO 21
<211> LENGTH: 585
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

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<210> SEQ ID NO 22
<211> LENGTH: 1290
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

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<210> SEQ ID NO 23
<211> LENGTH: 294
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

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atggcatcca gccctgagcc cgctgtggcc aagggaggct tcctgagctt ccttgaggcc 180

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aacatgttca gcgtcatcat ccccatgtgc ctggtacttc tgctcctggc gctcatcctg 240

cccctgctct tctacctccg aaaacgcaac aagacgggca agcatgacgt ccag 294

<210> SEQ ID NO 24
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 24

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<210> SEQ ID NO 25
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 25

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<210> SEQ ID NO 26
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 26

gcctcgagtt ccgcgtcact ggggccctg 29

<210> SEQ ID NO 27
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 27

actagtttac tacttatcgt cgtcatcctt gtaatcggcc acacggaggt aggg 54

<210> SEQ ID NO 28
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 28

tgctcgaggc cactgagcct tacaatgctg cc 32

<210> SEQ ID NO 29
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 29

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<210> SEQ ID NO 30
<211> LENGTH: 22

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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 30

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<210> SEQ ID NO 31
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 31

ggtctagatc ataatttact tcatcc 26

<210> SEQ ID NO 32
<211> LENGTH: 26
<212> TYPE: DNA
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<400> SEQUENCE: 32

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<400> SEQUENCE: 33

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<220> FEATURE:
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<400> SEQUENCE: 35

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<400> SEQUENCE: 37

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<210> SEQ ID NO 42
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<213> ORGANISM: Artificial
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<223> OTHER INFORMATION: phycoerythrin (PE)-conjugated E7 peptide

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Arg Ala His Tyr Asn Ile Val Thr Phe
1 5

<210> SEQ ID NO 43
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 43

Ile Leu Ser Asn Leu Ser Phe Pro Val
1 5

<210> SEQ ID NO 44
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 44

Leu Leu Phe Gly Ser Ile Val Ala Val
1 5

<210> SEQ ID NO 45
<211> LENGTH: 9
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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 45

Leu Ile Leu Pro Leu Leu Phe Tyr Leu
1 5

What is claimed is:

1. A recombinant *Listeria* strain comprising a recombinant nucleotide sequence encoding a recombinant polypeptide, said recombinant polypeptide comprising a fragment of a High Molecular Weight Melanoma-Associated Antigen (HMW-MAA) protein fused to a listeriolysin (LLO) oligopeptide, an ActA oligopeptide, or a PEST oligopeptide, wherein said nucleotide sequence encoding said fragment is selected from the group consisting of SEQ ID NOs: 21, 22 or 23.

2. The recombinant *Listeria* strain of claim **1**, wherein said HMW-MAA protein is a human HMW-MAA protein.

3. An immunogenic composition comprising the recombinant *Listeria* strain of claim **1**, or claims **5-7**.

4. The immunogenic composition of claim **3**, further comprising an adjuvant, cytokine, chemokine, or combination thereof.

5. A recombinant *Listeria* strain comprising a recombinant nucleotide sequence encoding a recombinant polypeptide, said recombinant polypeptide comprising a fragment of a High Molecular Weight Melanoma-Associated Antigen (HMW-MAA) protein fused to a listeriolysin (LLO) oligopeptide, an ActA oligopeptide, or a PEST oligopeptide, wherein said nucleotide sequence encodes SEQ ID NO: 21.

6. A recombinant *Listeria* strain comprising a recombinant nucleotide sequence encoding a recombinant polypeptide, said recombinant polypeptide comprising a fragment of a High Molecular Weight Melanoma-Associated Antigen (HMW-MAA) protein fused to a listeriolysin (LLO) oligopeptide, an ActA oligopeptide, or a PEST oligopeptide, wherein said nucleotide sequence encodes SEQ ID NO: 22.

7. A recombinant *Listeria* strain comprising a recombinant nucleotide sequence encoding a recombinant polypeptide, said recombinant polypeptide comprising a fragment of a High Molecular Weight Melanoma-Associated Antigen (HMW-MAA) protein fused to a listeriolysin (LLO) oligopeptide, an ActA oligopeptide, or a PEST oligopeptide, wherein said nucleotide sequence encodes SEQ ID NO: 23.

8. The recombinant *Listeria* strain of claim **1**, wherein said recombinant *Listeria* strain is a recombinant *Listeria monocytogenes* strain.

9. The recombinant *Listeria* strain of claim **1**, wherein said recombinant *Listeria* strain has been passaged through an animal host.

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